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Journal of

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VOLUME 18 FEBRUARY, 1959 NUMBER 1

CONTENTS

Page

Effect of Plane of Nutrition Upon Reproductive Performance of Boars. R. H. Dutt and C. E. Barnhart 3

Implanted and Oral Stilbestrol for Fattening Steers. C. C. O'Mary, A. E. Cullison and J. L. Carmon 14

Effects of Full and Restricted Winter Rations on Dams and Summer Dropped Suckling Calves Fed Different Rations. R. C. Hammes, Jr., R. E. Blaser, C. M. Kincaid, H. T. Bryant and R. W. Engel 21

Supplements to Silage Rations for Fattening Yearling Steers. L. S. Pope, R. D. Humphrey, D. F. Stephens, L. E. Walters and V. G. Heller 32

The Effects of Two Levels of Energy and Seasons on Reproductive Phenomena of Gilts. J. W. Gossett and A. M. Sorensen, Jr. 40

A Comparison of Embryo Survival in Gilts Slaughtered Twenty-Five Versus Forty Days After Breeding. J. W. Gossett and A. M. Sorensen, Jr. 48

Certain Semen Characteristics and Their Relation to the Reproductive Performance of a Purebred Hereford Herd. C. E. Lindley, G. T. Easley, J. A. Whatley, Jr. and Doyle Chambers 55

Studies on the Use of Hormones in Lamb Feeding. I. Production Performance. R. J. Davey and G. H. Wellington 64

Studies on the Use of Hormones in Lamb Feeding. II. Tissue Assays and Physiological Effects. R. J. Davey, D. T. Armstrong and W. Hansel 75

A Study of Stillbirths in a Herd of Range Cattle. R. R. Woodward and R. T. Clark 85

Relation of Heat Tolerance Determinations to Productivity in Beef Cattle. E. H. Vernon, R. A. Damon, Jr., W. R. Harvey, E. J. Warwick and C. M. Kincaid 91

The Effect of Carcass Grade, Weight and Classification Upon Boneless Beef Yield. D. H. Kropf and R. L. Graf 95

The Influence of Three-Week Weight on Eight-Week Weight of Pigs Weaned at Three Weeks of Age. W. S. Teter and L. E. Hanson .. 104

Body Measurements in Relation to Beef Type and to Certain Carcass Characteristics. G. M. Tallis, E. W. Klosterman and V. R. Cahill 108

Page

An Evaluation of the Antipyrine Dilution Technique for the Determination of Total Body Water in Ruminants. W. N. Garrett, J. H. Meyer and G. P. Lofgreen 116

The Rate of Fat Deposition in Four Lines of Swine. T. L. Noffsinger, F. N. Andrews and V. L. Anderson 127

The Comparative Effects of Urea, Uramite, Biuret, Soybean Protein, and Creatine on Digestion and Nitrogen Metabolism in Lambs. G. C. Anderson, G. A. McLaren, J. A. Welch, C. D. Campbell and G. S. Smith 134

The Effect of Selenium on Reproduction in Swine. R. C. Wahlstrom and O. E. Olson 141

The Chemical and Physical Characteristics of Various Pork Ham Muscle Classes. E. J. Briskey, R. W. Bray, W. G. Hoekstra, P. H. Phillips and R. H. Grummer 146

The Effect of Various Levels of Exercise in Altering the Chemical and Physical Characteristics of Certain Pork Ham Muscles. E. J. Briskey, R. W. Bray, W. G. Hoekstra, R. H. Grummer and P. H. Phillips 153

Blood Changes of Cattle During Bloat and Effects of the Alfalfa Forage *In Vitro*. H. D. Jackson, R. A. Shaw, W. R. Pritchard and B. W. Hatcher 158

The Effect of Exogenous Progesterone on Formation and Maintenance of the Corpora Lutea and on Early Embryo Survival in Pregnant Swine. H. G. Spies, D. R. Zimmerman, H. L. Self and L. E. Casida 163

The Effect of Exhaustive Exercise and High Sucrose Regimen on Certain Chemical and Physical Pork Ham Muscle Characteristics. E. J. Briskey, R. W. Bray, W. G. Hoekstra, P. H. Phillips and R. H. Grummer 173

Estrogen Excretion by Cows Following Oral Administration of Diethylstilbestrol. R. M. Melampy, J. Gurland and J. M. Rakes 178

The Influence of Aluminum and Zinc Upon the Absorption and Retention of Calcium and Phosphorus in Lambs. A. Thompson, S. L. Hansard and M. C. Bell 187

The Effect of Tranquilizers, Diethylstilbestrol and Oxytetracycline Alone and in Combination on Performance of Steers. W. C. Sherman, W. H. Hale, W. M. Reynolds and H. G. Luther 198

Influence of Fistulation on the Digestibility of Feeds by Steers. D. Drori and J. K. Loosli 206

Some Carcass Characteristics of Swine in the Eighth Generation of Production Under Four Combinations of Full and Limited Feeding. G. E. Brunstad and S. H. Fowler 211

Failure to Detect Residual Estrogenic Activity in the Edible Tissues of Steers Fed Stilbestrol. E. J. Umberger, J. M. Curtis and G. H. Gass 221

The Effect of Diethylstilbestrol, Reserpine, Hydoxyzine, and Hygromycin on Growth and Carcass Quality of Lambs. T. W. Perry, M. Stob, F. N. Andrews and W. M. Beeson 227

Page

Sensitivity of Various Tissues of Holstein Calves to Tocopherol Intake. A. P. Grifo, Jr., H. D. Eaton, J. E. Rousseau, Jr., and L. A. Moore 232

Effect of Several Combinations of Grass and Grain in the Ration of Beef Steers on the Color Characteristics of Lean and Fat. H. B. Craig, T. N. Blumer and E. R. Barrick 241

Comparative Procedures for Measuring the Phosphorus Requirement of Cattle. A. D. Tillman, J. R. Brethour and S. L. Hansard ... 249

Oral and Parenteral Administration of Iron in the Prevention and Treatment of Baby Pig Anemia. D. E. Ullrey, E. R. Miller, D. R. West, D. A. Schmidt, R. W. Seerley, J. A. Hoefer and R. W. Luecke 256

Magnesium Requirement of the Pig. R. H. Mayo, M. P. Plumlee and W. M. Beeson 264

Cellulose Digestion *In Vitro* as a Measure of the Digestibility of Forage Cellulose in Ruminants. G. V. Quicke, O. G. Bentley, H. W. Scott and A. L. Moxon 275

Relative Value of Carotene from Alfalfa and Vitamin A from a Dry Carrier Fed to Lambs and Pigs. G. S. Myers, Jr., H. D. Eaton and J. E. Rousseau, Jr. 288

Prenatal Reproductive Performance in Gilts Fed Low Levels of Stilbestrol in a Legume-Free and an Alfalfa Ration. E. P. Young and H. S. Teague 298

Progesterone Content of Bovine Reproductive Organs and Blood During Pregnancy. R. M. Melampy, W. R. Hearn and J. M. Rakes 307

The Relationship of Certain Blood Constituents to Rate and Economy of Gain in Beef Cattle. R. L. Arthaud, A. B. Schultze, R. M. Koch and V. H. Arthaud 314

Estimates of Genetic and Phenotypic Parameters in Beef Cattle. II. Heritability Estimates from Parent-Offspring and Half-Sib Resemblances. R. C. Carter and C. M. Kincaid 323

Estimates of Genetic and Phenotypic Parameters in Beef Cattle. III. Genetic and Phenotypic Correlations among Economic Characters. R. C. Carter and C. M. Kincaid 331

Influence of Pelleting on the Utilization of Alfalfa. J. H. Meyer, R. L. Gaskill, G. S. Stoewsand and W. C. Weir 336

The Effect of Two Levels of Energy Intake on Reproductive Phenomena in Duroc Jersey Gilts. C. E. Haines, A. C. Warnick and H. D. Wallace 347

The Gonadotrophic Content of Pituitary Glands from Gilts at Two Stages of Early Pregnancy and on Two Levels of Energy Intake. C. E. Haines and A. C. Warnick 355

Growth Potential of Swine as Measured by Serum Alkaline Phosphatase. G. E. Combs, H. D. Wallace, W. L. Alsmeyer and M. Koger 361

Lignin and Methoxyl Groups as Related to the Decreased Digestibility of Mature Forages. G. V. Quicke and O. G. Bentley 365

The Storage Potential and Citric Acid Content of Fractionated Ejaculates of the Boar. G. Bialy and H. L. Self 374

Page

An Attempt to Induce Urinary Calculi Formation in Steers with the Effects on Certain Blood and Urine Constituents. C. J. Elam, W. E. Ham and I. A. Dyer 383

The Effect of Delayed Shearing of Ewes and Daytime Cooling of Rams on Late Spring Breeding Performance. J. V. Whiteman and K. I. Brown 392

The Effect of Estrogenic and Androgenic Compounds Upon Growth and Carcass Composition of Swine. G. W. Thrasher, T. W. Perry, F. N. Andrews, W. M. Beeson and M. Stob 399

The Effectiveness of Three Different Methods of Iron Administration to Young Pigs. M. E. Rydberg, H. L. Self, T. Kowalczyk and R. H. Grummer 410

The Effect of Pre-Partum Intramuscular Iron Treatment of Dams on Litter Hemoglobin Levels. M. E. Rydberg, H. L. Self, T. Kowalcyzk and R. H. Grummer 415

Significant Relationships in Pork Carcass Evaluation. I. Lean Cuts as Criteria for Live Hog Value. S. E. Zobrisky, D. E. Brady, J. F. Lasley and L. A. Weaver 420

Stilbestrol Feeding and Growth Hormone Stimulation in Immature Ruminants. A. W. Struempler and W. Burroughs 427

Performance of Crossbred Beef Cattle in the Gulf Coast Region. R. A. Damon, Jr., S. E. McCraine, R. M. Crown and C. B. Singletary 437

Pork Quality. I. Influence of Some Factors on Pork Muscle Characteristics. M. D. Judge, V. R. Cahill, L. E. Kunkle and W. H. Bruner 448

Reproduction in the Yearling Ewe as Affected by Breed and Sequence of Feeding Levels. I. Effects on Ovulation Rate and Embryo Survival. W. C. Foote, A. L. Pope, A. B. Chapman and L. E. Casida 453

Reproduction in the Yearling Ewe as Affected by Breed and Sequence of Feeding Levels. II. Effect on Fetal Development. W. C. Foote, A. L. Pope, A. B. Chapman and L. E. Casida 463

Proceedings of 50th Annual Meeting 475

News and Notes 512

EFFECT OF PLANE OF NUTRITION UPON REPRODUCTIVE PERFORMANCE OF BOARS[1]

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THE relation of nutrition to reproduction in farm animals has been extensively reviewed by Reid (1949) and Asdell (1949). Many of the studies have been related to the effects of specific dietary requirements rather than to level of nutrition.

Phillips and Andrews (1936) reported a retardation in the development of the testes and germinal epithelium in boars raised from weaning on a low plane of nutrition. Studies on semen production were not made. Mann and Walton (1953) reported that semen production in a mature bull was not harmed by a 90-day period of underfeeding in which there was rapid loss of weight. Filpse *et al.* (1953) reported a delay in appearance of viable sperm and poor initial quality semen in bulls raised on 70% of the recommended allowance of total digestible nutrients. Niwa (1955) reported a delay in time of ejaculatory capacity in boars making subnormal growth but no effect on semen production after 7 months of age. Lack of adequately controlled studies with farm animals makes it difficult to assess the effects of nutritional level upon the reproductive performance of the male.

Since boars produce a large volume of semen per ejaculate, belong to a multiparous species, and are amenable to serving an artificial vagina, they are well adapted to a study of the effects of plane of nutrition upon reproductive capacity.

The present study is an attempt to determine the influence of three planes of nutrition from weaning time upon such characteristics in Hampshire boars as growth, age at sexual maturity, semen production, and fertility.

Materials and Methods

Nine weanling Hampshire boars were divided into three groups and placed on feed July 14 at an average age of 60 days. Six of the boar pigs were litter-mate trios, and the remaining three were from two different litters by the same sire. Ages differed by only 3 days, and the weights ranged from 23 to 32 lb. One boar from each litter or sire group was assigned to each treatment group, so that the average group weights were approximately equal.

All groups were fed a ration consisting of yellow corn 74.7 lb., soybean oil meal (43%) 22.0 lb., ground limestone 1.0 lb., steamed bonemeal 1.0 lb.,

[1] This investigation is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

salt 0.5 lb., vitamin and antibiotic premix 0.5 lb., and trace mineral premix 0.3 lb. The calculated crude protein content of the ration was 16.1%. The three groups of boars were hand-fed at different planes of nutrition, as follows: (1) 100%, (2) 70%, and (3) 50% of the National Research Council's (1953) recommended daily nutrient allowances for growing boars. The boars were weighed every 2 weeks and feed adjustments were made accordingly. When weight differences appeared among the groups, the feed given to the restricted intake groups was based on the average weight of the boars within each group, and not on the amount of feed consumed by the boars on the 100% plane. The boars were kept in concrete floored pens at all times and the groups remained on their respective feeding levels for 312 days, when the study was terminated.

When the boars were 183 days old, they were checked twice a week for attainment of puberty. Boars were allowed access to a female in heat and were considered to have reached puberty when they mounted the female and ejaculated into an artificial vagina a sample of semen containing spermatozoa. The artificial vagina was a modified conventional double-jacket type containing warm water at 42–45° F. A pressure bulb was attached, and the semen was collected in a plastic bag attached by means of a rubber band.

After the initial period of training, in determining the age of puberty, the boars would mount without hesitancy a gilt secured in a holding crate and serve the artificial vagina. A weekly semen collection was taken from each boar by means of the artificial vagina for a three-week period prior to the breeding period which began April 16. At the end of the breeding period, on May 17, the boars were placed on what was intended to be a semen exhaustion study. An attempt was made to secure an ejaculate of semen in the artificial vagina daily from each boar for a period of 7 days. This was done in order to obtain a measure of libido and of semen quality from the boars under rather heavy usage.

As soon as an ejaculation of semen had been obtained, the volume was determined and the gelatinous material separated from the fluid portion by means of a small strainer. The percent of motile cells was estimated at this time by placing a drop of the fresh semen beneath a cover slip on a slide in a warming chamber and examining under both low and high power of the microscope. Sperm cell concentration was determined by standard counting procedure using a hemacytometer. Percentage of morphologically abnormal cells was calculated by counting the abnormally shaped cells in the same fields on the hemacytometer from which cell concentration was computed. To prevent clumping of sperm cells which frequently occurs upon standing, and which would make counting extremely difficult and produce questionable results, three drops of a 5% solution of sodium cyanide was added to a 15 ml. sample of semen as soon as the volume had been determined. This procedure prevented cell clumping, and although it stopped all cell movement it did not appear to affect the cells otherwise.

A group of 27 gilts was made available for study of fertility. Between April 16 and May 17 (9 to 10 months after start of experimental feeding) each boar in turn was bred to three gilts. The gilts were checked daily for heat and each boar was allowed to serve a gilt only one time on the first day of heat. All gilts were slaughtered 25 to 28 days after breeding. The reproductive tracts were removed and taken to the laboratory, where the embryos were dissected from the uterine horns and their number and condition recorded; the number of corpora lutea present on the ovaries was also recorded. In non-pregnant gilts the number of regressing corpora lutea was recorded. The percent of corpora lutea represented by normal embryos at 25 to 28 days of gestation was the criterion used to measure fertility of the boars.

Results of the study were examined statistically by analysis of covariance on initial weight of the boars. In the analysis of the fertility data the percentages of corpora lutea represented by normal embryos were transformed to angles (Snedecor, 1946). In order to correct for difference in number of corpora lutea among the gilts, each angle was given a statistical weight equal to the number of corpora lutea upon which it was based. Variance for boars within plane of nutrition was used as the uncontrolled variance in testing the effect of plane of nutrition on fertility. Individual boar regression coefficients for the semen data on day during the seven-day collection period were calculated. Deviations of the group regression coefficients from zero were tested for significance using the t-test. Difference between group regression values were tested for significance by analysis of covariance.

Results

Daily Gain and Final Weight. Average daily gains during the 312-day feeding period and final weights for the three groups of boars are given in table 1. Average daily gains for individual boars ranged from 1.00 to 1.29 lb.; from 0.57 to 1.21 lb. and from 0.56 to 0.90 lb. for the boars on the 100-, 70- and 50-% planes of nutrition, respectively. Individual final weights for the boars varied from 200 to 435 lb. Average daily gains were not found to be significantly different; however, average final weight of boars on the 50-% plane differed significantly ($P<.05$) from those on the 100-% plane.

Age and Weight at Puberty. Average age at time of puberty (first observed ejaculation of sperm cells) and average weight at puberty for the three groups of boars are also given in table 1. Age at puberty ranged from 201 to 234 days; however, the group means were not significantly different. Weight at time of puberty did differ significantly among the boar groups. Group averages for both the 70-% plane and the 50-% plane groups were significantly lower ($P<.01$) than that of the boars on the 100-% plane of nutrition. Weight at time of puberty varied from 190 to

TABLE 1. AVERAGE VALUES AND STANDARD ERRORS FOR DAILY GAIN, FINAL WEIGHT AND WEIGHT AND AGE AT PUBERTY FOR BOARS ON THREE PLANES OF NUTRITION

Items	Plane of nutrition[a]		
	100%	70%	50%
Av. daily gain, lb.	1.13± .08	0.85± .19	0.69± .11
Av. final weight, lb.	382± 29	293± 61	243± 37*
Av. age at puberty, day	203±2.5	212±6.4	219±8.6
Av. wt. at puberty, lb.	223± 20	171± 21**	135± 6**

[a] Three boars on each plane of nutrition.
* Significantly different from 100-% plane of nutrition at the 5% level.
** Significantly different from 100-% plane of nutrition at the 1% level.

260 lb., from 150 to 215 lb., and from 123 to 144 lb., for the boars on the 100-, 70- and 50-% planes of nutrition, respectively.

Semen Production During Weekly Collections. Averages and their standard errors for the various semen characteristics studied are given by boar groups in table 2. Total volume of semen produced was the only characteristic found to differ significantly ($P < .05$) among the three groups. Semen volume varied from 75 to 305 ml. for individual ejaculates. Volume of semen minus the gelatinous material did not differ significantly among the groups. The gelatinous material represented 15.3, 15.4 and 15.7% of the total ejaculate for the 100-, 70- and 50-% planes, respectively, and appeared to be unaffected by the treatment.

Sperm cell concentration ranged from 212,000 to 568,000 cells/mm^3 for individual ejaculates; however, averages among the boar groups were not significantly different.

Of the total sperm cells, it was estimated in all ejaculates that 70% or more were motile. There was no significant difference in estimated percent of motile cells among the treatment group means.

TABLE 2. AVERAGE VALUES AND STANDARD ERRORS FOR SEMEN PRODUCTION DURING THE WEEKLY COLLECTION PERIODS

Semen characteristics (Average per ejaculate)	Plane of nutrition		
	100%	70%	50%
Volume, ml.			
Total	173±24.2[a]	137±21.1	121± 9.5*
Minus gelatinous material	145±19.4	119±17.0	102± 7.9
Motile cells, %	84± 1.7	82± 2.2	84± 1.7
Sperm cell conc., 1,000/mm^3	378±26	417±40	398±21
Abnormal cells, %	6.5± 0.4	8.6± 0.8	7.5± 0.6

[a] Each value is the average of nine ejaculates (three weekly collections from three boars on each plane of nutrition).
* Significantly different from the 100-% plane of nutrition at the 5% level.

TABLE 3. FERTILITY RESULTS IN GILTS BRED BY BOARS ON THREE PLANES OF NUTRITION

Items	Plane of nutrition		
	100%	70%	50%
No. gilts bred	9	9	9
No. pregnant 25 to 28 days	7	8	7
Total no. corpora lutea (all gilts)	102	103	106
No. of normal embryos	56	65	59
No. of degenerate embryos	3	4	4
Percent of corpora lutea represented by normal embryos:			
All gilts bred	54.9	63.1	55.7
Pregnant gilts only	75.7	74.7	70.2

Morphologically abnormal cells varied from 5.2 to 12.3% for individual ejaculates. Treatment group means did not differ significantly in percent of abnormal cells.

Fertility Test. Summarization of the results of the fertility tests is shown in table 3. The percentage of corpora lutea represented by normal embryos was calculated for all gilts bred and for pregnant gilts only. Results of the analysis of covariance of the angular transformations for the percentage of corpora lutea represented by normal embryos on all gilts bred and for pregnant gilts only are given in table 4. After removing the variation due to replication (gilts within boars), the mean square among boars within plane of nutrition was still larger than that for plane of nutrition, and fertility of the boar groups did not differ significantly. A small number of degenerate embryos was found, representing 5.1, 5.8, and 6.3% of all embryos present in gilts bred to the boars on the 100-, 70- and 50-% planes of nutrition, respectively.

Seven-day Semen Study. Group means for the various semen characteristics studied during the seven-day collection period are given in table 5. Total volume and volume minus the gelatinous material were the only characteristics found to differ significantly among the boar groups. Boars on the

TABLE 4. ANALYSIS OF COVARIANCE FOR ANGULAR TRANSFORMATIONS OF CORPORA LUTEA REPRESENTED BY NORMAL EMBRYOS

Source of variations	All gilts bred		Pregnant gilts only	
	d/f	Mean square	d/f	Mean square
Plane of nutrition	2	1397.9	2	385.4
Boars within plane of nutrition	6	6148.2	6	684.1
Gilts within boars	18	8918.7	13	1243.7

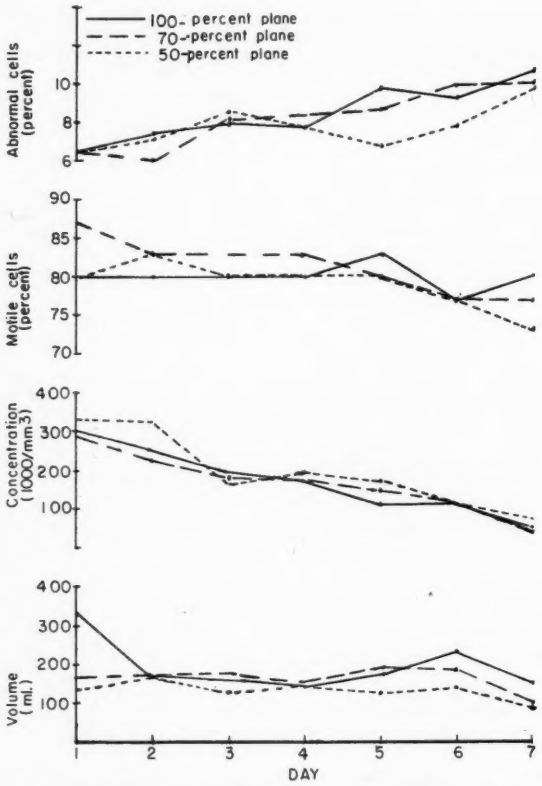

Figure 1. Average daily semen production from boars on three planes of nutrition during the seven-day collection period.

100-% plane of nutrition produced the largest volume of semen. Percent of motile cells, sperm cell concentration and percent abnormal cells were not significantly different among the treatment group. Sperm cell concentration for all three groups averaged about 50% of that during the weekly collection periods. Daily averages for total volume of semen, percent motile cells, sperm cell concentration and percent abnormal cells during the seven-day period are shown for each group of boars in figure 1.

Linear regression coefficients were calculated for the various semen characteristics on day for the seven-day test period and are given in table 6. During the period there was a decline in semen volume; however, the regression coefficient was significant ($P < .05$) only for volume minus the gelatin-

TABLE 5. AVERAGE VALUES AND STANDARD ERRORS FOR SEMEN PRODUCTION DURING THE SEVEN-DAY COLLECTION PERIOD

Semen characteristics (Average per ejaculate)	Plane of nutrition		
	100%	70%	50%
Volume, ml.			
Total	193±15.4[a]	163±10.7	137±9.7**
Minus gelatinous material	161±12.9	137± 9.1*	110±7.9**
Motile cells, %	79± 1.3	81± 1.2	79±1.2
Sperm cell conc., thousand/mm^3	173± 1.9	168± 1.9	195±2.1
Abnormal cells, %	8.5± 0.2	8.5± 0.4	7.8±0.4

[a] Each value is the average of 21 ejaculates—seven daily collections from three boars on each plane of nutrition.
* Significantly different from the 100-% plane of nutrition at the 5% level.
** Significantly different from the 100-% plane of nutrition at the 1% level.

ous material for boars on the 50-% plane. There was a significant decline ($P < .01$) in sperm cell concentration in all treatment groups. Coded regression coefficients ranged from —34.69 to —41.95 for the three groups; i.e., there was a reduction in average sperm cell concentration ranging from 34,690 to 41,950 sperm cells/mm^3 for each successive daily collection. A daily decline in percent motile cells was found. This was highly significant for boars on the 70-% plane and significant for those on the 50-% plane. There was a highly significant increase in percent of abnormal cells for the boars on the 100-% and 70-% planes of nutrition.

The nine regression coefficients for each semen characteristic studied were analyzed by analysis of covariance on initial weight; sperm cell concentration was the only one for which the differences among the three treatment groups were significant ($P < .05$). The regression coefficient for concentration was lowest on the 70-% plane.

TABLE 6. WITHIN PLANE OF NUTRITION LINEAR REGRESSION COEFFICIENTS FOR SEMEN CHARACTERISTICS ON DAY DURING THE SEVEN-DAY COLLECTION PERIOD

Semen characteristics	Plane of nutrition		
	100%	70%	50%
Volume, ml.			
Total	—13.04	—3.81	—8.09
Minus gelatinous material	—10.24	—3.09	—7.38*
Sperm cell conc., 1,000/mm^3	—41.21**	—34.69**	—41.95**
Motile cells, %	—0.7	—1.67**	—1.19*
Abnormal cells, %	.64**	.53**	.33

* Regression coefficient differs significantly from zero at the 5% level.
** Regression coefficient differs significantly from zero at the 1% level.

Discussion

The different planes of feed intake were effective in altering rate of growth of the boars as indicated by average weight at time of puberty and average final weight. However, there was no clear-cut indication that the reproductive capacity of the boars had been affected by the treatments. There was greater variation in gain among the boars on the 70- and 50-% planes than among those on the 100-% plane. The final weights ranged from 335 to 435 lb., from 205 to 410 lb., and from 200 to 310 lb. for the boars on the 100-, 70- and 50-% planes, respectively. The boars were group fed, and it became apparent that unequal feed consumption within the lower intake groups was probably responsible for part of the variation in growth. It is assumed that the fastest gaining boars within the low plane groups consumed a greater proportion of the allotted feed; however, other factors such as differences in efficiency of feed utilization could also account for part of the within-group variation in rate of gain. The large variation in rate of gain encountered in the groups on restricted feed intake emphasizes the need for individual feeding.

Ejaculation of sperm cells occurred at a later age in the boars on the low plane of nutrition, but the differences were not significant. A greater difference in age of puberty may have actually existed than was revealed in this study. Routine checking for ejaculation of sperm was not started until the average age of the boars was 183 days. If it is assumed that a period of training is necessary before boars will serve the artificial vagina, then perhaps the boars on the higher plane of nutrition were penalized, since they would not have had the advantage of as long a training period as did the boars that attained puberty at a later age. It is of interest, however, that the capacity to ejaculate sperm cells was established in the boars on the 50-% plane at an average age of 219 days. This suggests that development and functional activity of the genitalia of the male pig were relatively unaffected by the levels of feeding employed. The effect of feed intake on semen production in this study is similar to that reported by Niwa (1953). Wiggins *et al.* (1951) reported that the average age at onset of puberty in several lines of inbred boars ranged from 171 days to 217 days of age.

Fertility for all groups may appear to be somewhat low; however, fertility was based upon 25- to 28-day embryos and not on fertilized ova. The percentage of corpora lutea represented by normal embryos at 25 to 28 days in gilts bred by the three groups of boars does not differ greatly from that reported by Robertson *et al.* (1951) and Warnick *et al.* (1951). It is possible that a higher conception rate would have resulted if the gilts had been bred twice during the heat period (Weaver and Bogart, 1943). There was no evidence that plane of nutrition had any differential effect on embryo mortality to 25 to 28 days of gestation.

Since there was marked variation in individual boar gains within the lower intake groups, semen production for the weekly collections and fertility results were compared for the three fastest gaining boars and the three slowest gaining boars in the study. Included among the three fastest gaining boars were two from the 100-% plane and one from the 70-% plane. Among the three slowest gaining boars, two were from the 50-% plane and one from the 70-% plane. The average final weights of the boars in this grouping were 407 and 206 lb., respectively. In the gilts bred by the three fastest gaining boars 54.6% of the corpora lutea were represented by normal embryos, and in the gilts bred to the slowest gaining boars 57.1% were represented by normal embryos. Analysis of the semen data according to this grouping is not shown in the text; however, total volume was the only characteristic found to differ significantly ($P < .05$).

Since Rodolfo (1934) and McKenzie *et al.* (1938) reported a decline in semen production when boars were used daily, it is of interest to observe the changes in semen production by the treatment groups when under heavier usage. During the breeding period each boar was allowed approximately two services per week, which is not considered heavy usage for boars approaching one year of age. The seven-day semen collection period afforded the opportunity to determine whether any of the treatment groups tended to show greater changes in semen production during a period of relatively heavy usage. It is noteworthy that group averages for the various semen characteristics for the seven-day period were not greatly different from the weekly averages given in table 2. The one exception is for concentration which was lower for all treatment groups during the seven-day period.

Time-series regression coefficients reveal that changes in the various semen characteristics during the seven-day period were no greater for the groups on the restricted feed intake than for those on the 100-% plane. A highly significant decline in sperm cell concentration was found for all groups. Semen volume declined, but this was significant only for the 50-% plane for volume minus gelatinous material. Thus, it appears that for year-old boars under daily service the accessory organs are able to maintain semen volume, but the supply of sperm cells tends to become reduced.

Analysis of covariance of the regression coefficient for the various semen charactertistics showed a significant differencc between boar groups for only sperm cell concentration. Here there was no definite trend associated with plane of nutrition, since the lowest rate of decline occurred in the 70-% plane with the 100- and 50-% planes showing higher but similar rates of decline. Differences between boar groups in rate of change for estimated percent motile cells and for percent abnormal cells were not significant.

The seven-day semen study also provided some information regarding effect of plane of nutrition on libido. All boars served the artificial vagina each day during the seven-day period; however, it was frequently observed that the boars on the lower planes of nutrition displayed greater interest in

serving. This fact was more noticeable during the hot part of the day and for that reason collections during the seven-day test period were taken between 6 and 8 a.m. There was no indication that the restricted feed intake had any detrimental effects on libido.

The data indicate that the development and function of the reproductive system of the boar are rather resistant to effects of under-feeding. An attempt was made to supply all the necessary dietary requirements, but in restricted amounts. Although body growth and size were retarded and time of onset of puberty delayed in the restricted-intake groups, no deleterious effect on semen volume, sperm density, motility, cell morphology or fertility of the boars was observed.

Summary

Three groups of three weanling Hampshire boars were placed on 100%, 70% and 50% of the National Research Council's recommended T.D.N. intake for growing boars, and the effect upon growth, age at puberty and reproductive performance were studied. The boars remained on their respective nutritional planes for 312 days. Average daily gains and final weights were 1.13 and 382 lb., 0.85 and 293 lb., and 0.69 and 243 lb., respectively, for the three groups. Average ages at puberty were 203, 212 and 219 days, and average weights were 223, 171 and 135 lb., respectively. Weight but not age at puberty was significantly different among the boar groups on the different planes of nutrition.

Boars on the higher plane of nutrition produced larger semen volume, but motility, sperm cell concentration and percent morphologically abnormal cells were not significantly affected. During weekly semen collections average semen volumes were 173, 137 and 121 ml., respectively. Average sperm counts were 378,000; 417,000 and 398,000 cells/mm^3, respectively. Average motility was above 80% for all groups, and percent abnormal cells ranged from 6.5 to 8.6. The proportion of total semen volume composed of gelatinous material was 15.3, 15.4 and 15.7% for the three groups and appeared to be unaffected by nutritional treatment.

Fertility levels of the boars, using as a criterion the percent of corpora lutea represented by normal embryos in gilts at 25 to 28 days of gestation, were 54.9, 63.1 and 55.7%, respectively, for the three groups. For pregnant gilts only the percentages were 75.7, 74.7 and 70.2, respectively. Fertility did not differ significantly among the boar groups.

Time-series regression coefficients calculated on the various semen characteristics during a seven-day collection period revealed daily changes which were of similar magnitude for all treatment groups. Concentration was the only characteristic for which the group regressions differed significantly ($P < .05$). Restricting the plane of nutrition did not adversely affect libido of the boars as judged by their breeding behavior during a seven-day test period.

This study demonstrates that development and functional capacity of the reproductive system of boars are relatively resistant to the effects of underfeeding which resulted in reduced body growth and size.

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IMPLANTED AND ORAL STILBESTROL FOR FATTENING STEERS [1, 2, 3, 4]

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THE implantation of 36 mg. of stilbestrol has been shown to produce significant increases in gains in steers fed a ration high in blackstrap molasses in drylot (O'Mary *et al.*, 1953, 1956). Implants of 24 mg. per steer significantly increased gains of steers on pasture (O'Mary and Cullison, 1956). No undesirable side effects were noted except for a depression of the loin in some instances. Feeding of 10 mg. of stilbestrol per head daily has been effective in increasing rate of gain and feed efficiency with no observable side effects in steers on a grain ration (Burroughs *et al.*, 1954).

The objectives of the experiments herein reported were to determine (1) if the feeding of 10 mg. of stilbestrol per head daily would produce significant increases in gains of steers fed high levels of blackstrap molasses; (2) if the implantation of levels below 36 mg. would produce significant increases in gains on such rations without undesirable side effects; (3) if livers from steers either implanted with or fed stilbestrol contain significant estrogenic activity; (4) the effects of feeding stilbestrol to steers or reimplanting steers with stilbestrol in drylot which had been previously implanted with the hormone while on pasture; and, (5) the effects of a combination of orally administered and implanted stilbestrol for fattening steers in drylot.

Experimental Procedure

Experiment I. Two groups each of 10 steers were full-fed on the same ration, consisting of blackstrap molasses, cottonseed hulls, dehydrated alfalfa meal, cottonseed meal, defluorinated phosphate and salt, except that one group received in addition 10 mg. of stilbestrol per head daily (table 1). These steers mostly of medium grade were allotted at random to treatment. The duration of the experiment was 149 days, from December, 1954 to May, 1955.

Experiment II. On December 2, 1955, 40 steers were blocked according to weight and breed and randomly allotted within block to four groups. These groups were fed identical rations consisting of the same ingredients as in Experiment I. The treatments were (1) control; (2) 10 mg. stilbestrol

[1] The stilbestrol pellets (Capette) were furnished by Wick and Fry, Inc. through the courtesy of W. S. Davidson.

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fed daily; (3) 12 mg. stilbestrol implanted; and, (4) 24 mg. implanted. Five of the steers in group 3 were reimplanted with 36 mg. of stilbestrol 27 days prior to slaughter. When the animals were slaughtered two livers were taken at random from each treatment group, the two samples being taken from the reimplanted animals in group 3. The livers were frozen and stored until used.

Bioassay of the livers for estrogenic activity was made with 21-day-old intact female mice. Four groups of mice were fed for 10 days diets containing liver obtained from each of the four steer groups. The procedure used was that described by Preston *et al.* (1956). The diet contained 80% of the dried liver and 20% of a basal diet. A fifth group of mice was fed a ration containing livers of the control steers to which was added 0.005 mcg. of stilbestrol per gram of diet. The uteri of the mice were dissected and weighed as described by Turner (1956). Uterine weights were analyzed statistically by analysis of variance.

Experiment III. Forty-eight steers most of good grade were used in this study. Twenty-four of these steers had been implanted with 24 mg. of stilbestrol each, 211 days prior to the start of this experiment. The other 24 steers had served as the controls. Six of the treated and six of the control steers were randomly allotted to the following four feeding groups: (1) Molasses, control; (2) molasses, stilbestrol-fed; (3) corn, control; and (4) corn, stilbestrol-fed. These groups with the detailed rations are shown in table 3. Three steers selected at random from each of the sub-groups of six were implanted with 24 mg. of stilbestrol. A statistical analysis was made to determine (1) if there were any differences between corn and molasses or (2) if the stilbestrol at the 10 mg. level had any effect when fed with either ration and (3) if there was a ration-hormone interaction. Also previous treatment, reimplantation, and the possible interactions were studied.

Results and Discussion

Experiment I

Daily Rate of Gain and Feed Consumption. The average daily gain of the stilbestrol fed steers shows only a slight (non-significant) increase over the control steers when both groups were fed a high-molasses ration. The average daily feed consumption was practically identical in the two groups.

Carcass Grades, Slaughter Grades and Dressing Percent. The average slaughter grade of both groups of steers was low good. The respective carcass grades tended to be a bit lower, both groups grading high standard. The dressing percent of steers in the hormone-fed group was slightly higher but was not significantly different from that of the controls.

Experiment II

Feed Consumption. The rations and the amounts of each feed consumed per head daily are shown in table 2. The total feed intake was essentially

the same for each group. With these rations there were no indications that stilbestrol caused any stimulation of appetite although one might speculate that the appetite was already stimulated by the molasses since the total feed intake was so great in the control group. Daily dry matter intake was 14.5% in excess of requirements as calculated by Morrison's standards.

Rate of Gain. The average daily gains in pounds (table 2) made by the steers over the 123-day period were (1) control, 2.14; (2) DES fed, 10 mg., 2.22; (3) 12 mg. DES implanted, 2.12; and (4) 24 mg. DES implanted, 2.33. The gains were analyzed at various times throughout the experiment

TABLE 1. THE EFFECTS OF ORAL STILBESTROL ADMINISTRATION TO STEERS ON A HIGH-MOLASSES RATION

Item	Control	Stilbestrol-fed[a]
No. of animals	10	10
Av. initial weight, lb.	681.6	670.7
Av. daily gain, lb.[b]	1.93	2.02
Av. daily feed consumption, lb.		
Molasses	10.87	11.17
Cottonseed hulls	13.06	13.37
Cottonseed meal	3.91	3.91
Dehydrated alfalfa meal	2.00	2.00
Defluorophos	.10	.10
Salt	.05	.05
Total	29.99	30.60
Av. slaughter grade[c]	12.6	13.1
Av. carcass grade[c]	12.3	12.3
Av. dressing percent[d]	55.0	56.3

[a] Ten mg. stilbestrol per head daily.
[b] Standard deviation, 0.35 lb.
[c] 12=high-standard and 13=low-good.
[d] Based on lot weights and warm carcass weights.

but showed no significant differences at any time. Since in previous work (O'Mary *et al.*, 1953) 36 mg. stilbestrol implants resulted in a significant increase in gains with steers receiving high levels of molasses, these results might be interpreted to mean that neither the 12 nor 24-mg. implant nor the 10 mg. that were fed were sufficient to give optimum response on this type of ration. The mean daily gains of the steers receiving the 24-mg. implant exceeded the average daily gain of those receiving the 12-mg. implant. The average daily gain of steers fed stilbestrol was intermediate to that of the two implanted groups. This would seem to indicate that over a 123-day period a dosage level of between 12 and 24 mg. of stilbestrol implanted would be equivalent to 10 mg. of the hormone fed.

Dressing Percent and Carcass Grade. Dressing percentages were calculated for each treatment group. The carcasses were graded to within one-third of a grade by a federal grader. The results indicated that there were no significant differences between treatments in either dressing percentages or carcass grades. In no case was the mean of any of the treated groups less

TABLE 2. EFFECTS OF ORAL OR IMPLANTED STILBESTROL IN STEERS FED HIGH LEVELS OF BLACKSTRAP MOLASSES

Item	Control	10 mg. fed	12 mg. implanted	24 mg. implanted
No. of animals	10	10	10	10
Av. initial weight, lb.	639.9	632.7	644.1	619.3
Av. final weight, lb.	903.0	905.9	904.6	906.4
Av. daily gain, lb.[a]	2.14	2.22	2.12	2.33
Av. daily feed consumption, lb.				
Blackstrap molasses	12.92	12.91	12.90	12.79
Cottonseed hulls	9.96	9.92	9.96	9.96
Dehydrated alfalfa meal	1.00	1.00	1.00	1.00
Cottonseed meal	4.16	4.14	4.16	4.16
Defluorophos salt (2:1 ratio)	.15	.15	.15	.15
Total	28.19	28.12	28.17	28.06
Av. carcass grade[b]	11.0	11.4	11.8	11.0
Av. dressing percent[c]	55.8	55.9	56.6	55.9

[a] Standard deviation 0.36 lb.
[b] 11=standard and 12=high-standard.
[c] Based on lot weights and warm carcass weights.

than the control group in either dressing percent or carcass grade. The mean values of each of the groups are shown in table 2.

Side Effects. The only undesirable side effects noted were increased teat length and some raised tailheads.

Bioassay of Livers. Preston *et al.* (1956) had indicated that in one of their experiments liver tissue of stilbestrol-fed cattle produced heavier mouse uterine weights than either lean or fat tissue; yet, in another experiment the fat tissue produced the heavier mouse uterine wights. In the present experiment the average uterine weights of immature mice on diets including liver from each of the four treatment groups were obtained. The results agree fairly closely with those reported by Preston *et al.* (1956) and Turner (1956). The average uterine weights of mice on the diets of liver from the various stilbestrol treatment groups did not differ significantly from the controls.

A fifth group of mice was fed liver from the control steers to which was added 0.005 mg. of stilbestrol per gm. of diet. This group was included to serve as a positive control. However, the average uterine weight was 12.9

TABLE 3. UTERINE WEIGHT RESPONSE OF IMMATURE FEMALE MICE FED LIVER FROM STEERS RECEIVING STILBESTROL

Items	Control steers	Control+ DES[a]	DES fed	DES implanted	
				24 mg.	36 mg.
No. of mice	6	7	6	8	7
Av. uterine weight, mg.[b]	11.7	12.9	10.6	9.9	12.4

[a] Stilbestrol, 0.005 mcg. added per gm. of diet.
[b] Standard deviation, 3.5 mg.

mg. for this group and 11.7 mg. for the negative control. The difference between the two groups was not statistically significant, indicating that the liver from the various hormone treated steer groups did not contain estrogenic activity exceeding 0.005 mcg. per gram of mouse diet. This was based on uterine weight response of intact 21-day-old mice (table 3).

Experiment III

In experiments I and II the feeding of stilbestrol failed to stimulate significant increases in daily gains in steers on a high blackstrap molasses ration. This was further tested in experiment III. The results are presented in table 4. Again, the feeding of stilbestrol failed to stimulate increased gains of treated over control steers fed a high molasses ration. Also, the feeding of 10 mg. of stilbestrol per head daily to steers receiving a corn ration failed to increase gains over control steers in this test (table 4). Steers on the corn ration made significantly greater gains than those on the blackstrap molasses ration.

TABLE 4. STILBESTROL IN RATIONS FOR FATTENING STEERS [a]
(139 days)

Ration and treatment	Molasses C. S. hulls Supplement no. 1	Molasses C. S. hulls Supplement no. 1 Stilbestrol-fed	Corn Hay Supplement no. 2	Corn Hay Supplement no. 2 Stilbestrol-fed
No. of animals	12	11	12	12
Av. initial weight, lb.	745.8	760.7	742.0	757.5
Av. final weight, lb.	1027.4	1039.6	1076.4	1079.2
Av. total gain, lb.	281.6	278.9	334.4	321.7
Av. daily gain, lb.[b]	2.03	2.01	2.41	2.31
Av. daily feed consumption, lb.				
Molasses	13.62	13.55	.....	.....
Cottonseed hulls	9.38	9.38	.....	.....
Supplement (no. 1)	6.00	6.00	.....	.....
Corn	.....	.....	13.22	13.28
Alfalfa hay	.....	.....	9.93	10.01
Supplement (no. 2)	.....	.....	1.15	1.15
Stilbestrol, mg.	.....	10.0	.....	10.0
Av. feeder grade[c]	14.2	14.1	13.8	14.4
Av. slaughter grade[c]	13.9	13.9	14.9	15.5
Av. carcass grade[c]	13.4	12.9	14.7	15.2
Av. dressing percent[d]	56.8	56.0	58.7	58.8

[a] One-half of each group had been implanted with 24 mg. of stilbestrol at the start of a grazing period 211 days prior to start of the drylot feeding period; one-half of the previously implanted animals and one-half of the non-implanted animals in each group were implanted with 24 mg. of stilbestrol at the start of the feeding period. Performance on the basis of implant groups is given in table 5.

[b] Standard deviation, 0.38 lb.

[c] 13=low good; 14=average good; and 15=high good.

[d] Based on lot weights and warm carcass weights.

Supplement no. 1 contained alfalfa meal 1.0, cottonseed meal (41%) 4.85, defluorinated phos. 0.10 and salt 0.05. Supplement no. 2 contained cottonseed meal 1.00, and the same minerals as no. 1.

Stilbestrol implantations have resulted in increased rates of gains in steers being finished in drylot (O'Mary *et al.*, 1956) and in steers on pasture (O'Mary and Cullison, 1956). One of the objectives of this experiment was to determine if steers that had been under the influence of stilbestrol implants while on pasture would do as well as untreated steers when placed in drylot for finishing. In this test steers that were treated as long as 211 days prior to going into drylot did not do as well during the drylot feeding period as steers that had never been implanted (table 5). However, the steers which were reimplanted with 24 mg. of stilbestrol showed a stimulation in rate of gain, although it was not as great as in the group which was implanted for the first time as it went into the drylot feeding period.

TABLE 5. STILBESTROL IMPLANTS FOR FATTENING STEERS IN DRYLOT
(139 days)

Treatments	Not implanted on pasture or in drylot	Implanted on pasture,[a] not in drylot	Not implanted on pasture. Implanted in drylot	Implanted on pasture[a] and in drylot
No. of animals	11	12	12	12
Av. initial weight, lb.	763.5	754.3	700.5	788.8
Av. final weight, lb.	1056.3	1015.5	1053.5	1098.7
Av. total gain, lb.	292.8	261.2	353.0	309.9
Av. daily gain, lb.[b]	2.11	1.88	2.54	2.23
Av. feeder grade[c]	14.8	14.6	13.8	14.2
Av. slaughter grade[c]	14.6	14.4	14.7	14.5
Av. carcass grade[c]	15.2	14.5	13.9	13.8
Av. dressing percent[d]	57.5	58.0	57.7	57.4

[a] Implantation on pasture with 24 mg. of stilbestrol took place 211 days before the start of the drylot feeding period.
[b] Standard deviation, 0.38 lb.
[c] 13=low good; 14=good; and 15=high good.
[d] Based on lot weights and warm carcass weights.

In this test implants of as little as 24 mg. of stilbestrol per steer resulted in significant increases in daily gains of treated steers over control steers. The difference was 0.31 lb. per head daily in favor of the treated animals. In experiment II the difference between the hormone-implanted and the control steers was 0.19 lb. This was not a significant difference, but in both experiments the trends were similar.

In this test there were no significant treatment-ration interactions; thus, the effects of the stilbestrol implantations have been shown in table 5.

The slaughter grades, carcass grades and dressing percentages did not appear to be affected by the implantation of 24 mg. of stilbestrol per steer.

Another phase of experiment III involved a study of the effects of a combination of orally administered and implanted stilbestrol on the performance of steers being fattened in drylot. Steers which were implanted with 24 mg. of stilbestrol at the start of the feeding period and then were fed 10 mg. of the hormone daily in addition to the implant gained at the rate of 2.31 lb. per head daily as compared to 2.46 lb. per head daily for

those that received implants only and 1.95 lb. per head daily for those that were not implanted but were fed stilbestrol. The above differences, however, did not prove to be statistically significant.

Summary

Three experiments were conducted to determine the effects of oral and low level implants of stilbestrol in steers fed high levels of blackstrap molasses. In each of the three experiments the feeding of 10 mg. of stilbestrol per head daily failed to produce significant increases in gains of treated steers over the control steers. Carcass grades and dressing percentages were not significantly affected by treatments.

The implantation of as little as 24 mg. of stilbestrol per steer produced a significant increase in the average daily gains of treated steers over control steers in experiment III but not in experiment II, although the trend was in the same direction. The evidence indicates that for feeding periods of 120 to 150 days duration, 24 mg. of implanted stilbestrol will be more effective in increasing weight gains on rations involving high levels of molasses than either 12 mg. implanted or 10 mg. fed.

In experiment II estrogenic activity of livers from steers which received the hormone either orally or implanted was not significantly different from the controls based on uterine weight response of intact 21-day-old mice.

In experiment III steers which were implanted with stilbestrol while on pasture did not do as well when placed in drylot as steers that had not been previously implanted. A second implant as the steers were placed in drylot resulted in some stimulation of daily gains. However, those steers which were implanted for the first time as they were placed in drylot responded most favorably to treatment. There was no apparent advantage from the feeding of stilbestrol in conjunction with its use as implants in this study.

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EFFECTS OF FULL AND RESTRICTED WINTER RATIONS ON DAMS AND SUMMER DROPPED SUCKLING CALVES FED DIFFERENT RATIONS

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A REVIEW of recent literature reveals no pertinent data on summer calving as related to the rations required by the cow and her suckling calf during the winter. In areas where low temperatures prevent pasture growth for a 3- to 6-month season, high feeding costs and low or unprofitable calf gains are often associated with summer (July through September) calving. The high feed costs during the winter are attributed to labor and higher costs per unit of TDN from stored roughages and concentrates than from pastures.

Winter feed costs might be lowered by restricted feeding of dams and feeding high quality silage and hay to the calves. It is apparent that calves digest good quality forage about as effectively as mature ruminants. McArthur (1957) found that the dry matter digestibility of a given pasturage was 75.2% for cows as compared to 74.6% for Jersey calves that were 8 weeks old and weighed 120 lb. McMeekan (1954) reported that calves may be weaned when 8 weeks old or at a weight of 120 lb., and that they mature normally when fed good pasturage. He also reported that calves must have early access to pasture, as rumination starts as early as 7 days after birth. Preston (1957) found that the dry matter digestibility of grass fed to calves 3 to 10 weeks old was 74.6% and that milk feeding did not influence digestibility. According to Porter and Kesler (1954, 1957) alfalfa silage, hay, or combinations of hay and silage were equally satisfactory for calves from 6 days to 16 weeks of age. They also report that grass silage may be used as the sole forage for young dairy calves. Thomas *et al.* (1957) found subnormal gains for dairy calves fed alfalfa silage as compared with hay of the same crop, and that grain feeding did not alter this relationship.

This paper deals with winter feed rations when dams and their suckling calves were fed separate rations. The birth period of calves ranged from July through September.

Experimental Procedure

Two series of winter feeding experiments were conducted at the Northern Virginia Pasture Research Station using dams and their calves (bulls and

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heifers) from a pure bred Aberdeen-Angus breeding herd. All the dams in this herd dropped their first calf when 2½ years old during July, August, and September of 1951. This original herd supplied the experimental animals each year except in 1955, when one yearling bred heifer with her calf was assigned to each of the rations.

The dams had good pastures during the grazing season, but hay and/or silage was supplemented during November and continued until the dams and calves were assigned to the experimental rations in Mid-December.

Experiment I. 1952 [1] *and 1953.* In 1952, the feeding trial was conducted in 50′ × 250′ lots on a northerly slope with good drainage. In 1953 the lots were on a southerly slope and were 90′ × 250′. The lots did not have shelters during either year. The dams and calves were weighed on two consecutive days at the beginning and end of the experiment and at 28-day intervals between. The experiment was conducted for 54 days starting January 5 in 1952 and for 84 days starting December 4 in 1953. The feed was restricted in 1952, but in 1953 the dams and calves had all the feed they wanted. During this trial all dams with their calves had access to a given ration, except for grain which was fed to the dams.

The following rations were used during this experiment:

Ration of dam and calf	Years	
Alfalfa-orchardgrass silage	1952	1953
Alfalfa-orchardgrass (silage and hay)	1952	1953
Corn silage and alfalfa-orchardgrass hay	1952	1953
Alfalfa-orchardgrass (silage and hay) and ground ear corn	1952	....
Alfalfa-orchardgrass (silage and hay) and grain	1952	1953
Alfalfa-orchardgrass hay, alone	1952	1953
Corn silage, alfalfa-orchardgrass hay, and grain	....	1953

Animal assignments to the rations were based on the sex, age, and weight of the calves. In 1952 there were six rations; hence, six calves of the same sex and similar in age and weight were selected and one calf was then assigned at random to each of the rations. This was repeated with six other calf groups, making a total of seven calves per ration. Each dam accompanied her calf. This method of assigning animals was used for all trials. There were six calves and dams per ration in 1953.

Experiment II. 1955, 1956, and 1957. These experiments were conducted in fenced lots 90′ × 250′ on a uniform, well-drained southerly slope without shelter. A creep with 90 sq. ft. of floor space and 28 linear feet of manger was provided in each lot to feed different calf rations. During 1955 the calves had access to the dams' feeders which resulted in a few calves consuming a small amount of the silage and hay fed to the dams. However, feeder construction held this unmeasurable consumption to a very limited

[1] In all cases the year of the feeding trial refers to the passing winter season; i.e. 1952 refers to the 1951–1952 trial.

amount. In order to obtain accurate data on consumption by the calves in 1956 and 1957, the creeps were fenced to confine the calves to the creeps for 2 to 3 hours during the morning and evening feeding of the dams. The calves had access to the creeps at all times and *ad libitum* feeding of the ration constituents was practiced. The corn silage and hay not eaten by the calves was removed and weighed every two days.

The dams and calves were fed corn silage and alfalfa-orchardgrass hay for about 2 weeks before the experiment was started and were fed on their respective experimental rations for 4 days before they were weighed at the beginning of the experiment each year. This method adjusted for fill from a given ration to give more reliable live-weight data. Weights were obtained every 28 days during each trial. On weighing days, water was withdrawn at daybreak and all weighings were made in late morning, approximately 4 hours after feeding. The trials were conducted for 98, 91, and 91 days, starting December 10, 14, and 14 in 1955, 1956, and 1957, respectively.

The following rations were used during this experiment:

	Daily rations of dams		
Ration	Level	Dry matter	Ration for suckling calf
1	100%	Corn silage, 9.5 lb. Alf.-orch. hay, 14.4 lb.	(a) Corn silage and alf.-orch. hay
2	100%	Same as (1)	(b) Corn silage, alf.-orch. hay and grain mixture
3	75%	Corn silage, 7.1 lb. Alf.-orch. hay, 10.9 lb.	Same as (a)
4	75%	Same as (3)	Same as (b)
5	50%+ grain	Corn silage, 4.7 lb. Alf.-orch. hay, 7.2 lb. Grain mixture, 3.6 lb.	Same as (a)
6	50%+ grain	Same as (5)	Same as (b)
7	100%	Same as (1)	Grain mixture[1]
8	100%	Same as (1)	No supplement[1]

[1] Included during 1956 and 1957.

The corn silage and second to fourth cutting of alfalfa-orchardgrass hay fed the dams and calves were similar from the same source and the average composition of each of the experimental feeds is given in Table 1. To simplify the presentation of the data, the three rations fed the dams are termed 100%, 75%, and 50% rations. The 100% ration was based on the amount of feed the dams would consume in approximately 2 hours each morning and evening. By weekly dry matter adjustments, the dry matter fed each dam on a given ration was kept constant for all 3 years.

An analysis of variance of the data for all 3 years was made of the factorial rations 1 through 6 (three dam rations with each of two calf

rations). A separate analysis of variance was made during the last 2 years for rations 1, 2, 7, and 8 (four calf rations with dams on a 100% ration). The failure of the assigned calf groups to behave relatively the same made up the experimental error.

Animal assignments to the rations were made in the same manner as for Experiment I. There were six, six, and five calves and dams per ration in 1955, 1956, and 1957, respectively. The average weight of the calves or their dams for the rations did not differ significantly for this method of animal allotment.

This experiment was continued for 3 years because digestibility and palatability of forages vary from year to year, and including more animals also improved the reliability of the results.

TABLE 1. AVERAGE PERCENTAGE COMPOSITION OF THE RATION COMPONENTS (DRY BASIS)

Feed	Protein	Ether extract	Crude fiber	Ash	NFE
Corn silage	10.31	2.63	20.72	7.25	59.09
Hay[b]	17.38	2.22	27.23	8.55	44.62
Grain for dams[c]	20.78	3.72	8.06	3.15	64.32
Grain for calves[d]	16.25	3.41	7.94	2.78	69.62

[a] The corn produced about 60, 45, and 80 bushels per acre in 1954, 1955, and 1956, respectively.
[b] Alfalfa-orchardgrass mixture (65% alfalfa) field cured from 2nd, 3rd, and 4th harvest was excellent quality.
[c] Equal parts of ground ear corn, ground barley, and cottonseed meal (41% protein).
[d] Two parts each of ground ear corn, ground barley, and 1 part of cottonseed meal (41% protein).

Results

Experiment I. The rations for dams and calves and their daily gains during 1952 and 1953 are given in table 2. The daily liveweight gains of the calves during 1952 averaged 1.54 lb. for the hay ration with corn silage as compared to 1.31 to 1.56 lb. for rations of alfalfa-orchardgrass silage and/or hay. During 1953, the calves on rations with corn silage gained from 1.85 to 1.91 lb. daily as compared with lower daily gains ranging from 1.57 to 1.61 lb. for the alfalfa-orchardgrass (silage and/or hay) rations.

During 1952 the daily liveweight gains per dam for the rations ranged from —.06 to 0.97 lb. and for 1953 from 0.30 to 1.35 lb. Grain influenced the gains of the dams more in 1952 when added to a restricted ration than to a full ration in 1953. Dams fed alfalfa-orchardgrass as silage, hay, and both silage and hay gained 1.20, 0.30, and 0.68 lb. daily in 1953, respectively.

Experiment II. The average daily liveweight gains for the 3 years (table 3) show that the dams on a 100% ration gained 0.52 lb. as com-

pared to losses of 0.24 lb. and 0.37 lb. per head when fed the 75% and 50% rations, respectively. By July of each year, there were no differences between weights of dams that had been fed full or restricted rations.

The calf data (average of two calf rations, corn silage-hay, and corn silage-hay-grain) show daily gains of 1.97 lb., 1.95 lb., and 1.96 lb. per

TABLE 2. DAILY GAINS OF DAMS AND THEIR SUCKLING CALVES WHEN FED DIFFERENT RATIONS DURING 1952 AND 1953

Ration constituents	Dry matter daily		Daily gains			
			Dams		Calves	
	1952	1953	1952	1953	1952	1953
	lb.	lb.	lb.	lb.	lb.	lb.
Alfalfa-orchardgrass silage	22.0	31.6	0.25	1.20	1.37	1.60
Alfalfa-orchargrass hay						
2nd–4th cutting	24.9	31.6	0.29	0.30	1.45	1.57
Alfalfa-orchardgrass						
Hay	5.2	12.0	—0.06	0.68	1.31	1.61
Silage	18.2	19.6				
Alfalfa-orchardgrass						
Hay	4.3	9.5	0.53	1.09	1.51	1.58
Silage	17.5	19.6				
Ground ear corn	2.5	2.5				
Alfalfa-orchardgrass						
Hay	4.3	....				
Silage	17.5	....	0.97	....	1.56	....
Grain mixture[a]	2.5	....				
Corn silage	11.1	19.6	0.03	1.11	1.54	1.91
Alfalfa-orchardgrass hay	5.2	12.0				
Corn silage	....	19.6				
Alfalfa-orchargrass hay	....	9.2	....	1.35	....	1.85
Grain mixture[a]	....	2.5				

[a] 1-1-1-1 parts wheat, barley, ear corn, and cottonseed meal.

head for the 100%, 75%, and 50% dam rations, respectively. Thus, restricting the dams' ration did not influence the gains of the suckling calves.

Results during the 3 years in respect to the 100%, 75%, and 50% dam rations show that calves supplemented with corn silage and hay gained 1.77, 1.64, and 1.80 lb. as compared with 2.14, 2.17, and 2.10 lb. per head daily for calves supplemented with corn silage, hay, and grain (table 4). The calf gains within the two calf rations were not influenced by rations of the dam as interactions were not significant during any year nor for the combined data for the 3 years. Differences in the gains of the calves fed the corn silage-hay-grain ration were significantly higher than gains of the calves fed corn silage and hay.

TABLE 3. THE EFFECTS OF FULL AND RESTRICTED RATIONS FOR DAMS ON LIVEWEIGHT GAINS DURING THREE WINTER FEEDING PERIODS [a]

Items	100% Ration				75% Ration				50% Ration			
	1955	1956	1957	Av.	1955	1956	1957	Av.	1955	1956	1957	Av.
Av. initial weight, lb.[b]	974	1032	1053	1020	994	1024	1012	1010	928	966	1028	974
Av. final weight, lb.	1046	1066	1094	1069	993	977	995	988	917	916	987	940
Av. daily gain per dam, lb.[b]	0.73	0.37	0.45	0.52	—.01	—.52	—.19	—.24	—.11	—.55	—.45	—.37
Av. daiy gain per calf, lb.	1.88	1.98	2.04	1.97	1.74	1.95	2.15	1.95	1.84	1.89	2.14	1.96
Av. daily dry matter consumed per dam, lb.												
Corn silage	10.9	8.0	9.7	9.5	8.1	6.0	7.3	7.1	5.4	4.0	4.9	4.7
Hay	13.0	16.0	14.2	14.4	9.7	12.1	10.7	10.9	6.4	8.1	7.1	7.2
Grain	....	....	....	....	....	....	....	....	3.6	3.6	3.5	3.6
Total	23.9	24.0	23.9	23.9	17.9	18.1	18.0	18.0	15.4	15.7	15.5	15.5
Estimated TDN intake per dam, lb.												
Corn silage				6.3				4.7				3.1
Hay				8.1				6.1				4.1
Grain				....				....				2.9
Total				14.4				10.8				10.1

[a] There were 12 dams and calves per factorial treatment in 1955 and 1956 and 10 dams and calves in 1957.

[b] The differences in liveweight gains or losses were significant at the 1% level for all years as well as for the average values. The initial weights did not differ significantly.

TABLE 4. AVERAGE DAILY DRY MATTER CONSUMED AND DAILY GAINS OF SUCKLING CALVES AS INFLUENCED BY RESTRICTED FEEDING OF THE DAM [a]

Items	Dams 100% ration				Dams 75% ration				Dams 50% ration			
	1955	1956	1957	Av.	1955	1956	1957	Av.	1955	1956	1957	Av.
Calves fed forages												
Av. initial calf age, days	126	145	144	138	126	142	147	138	120	137	139	132
Av. initial weight, lb.	228	311	326	288	249	314	319	294	245	311	321	292
Av. final weight, lb.	393	463	503	453	391	466	484	447	407	468	505	460
Av. daily gain per calf, lb.[b]	1.58	1.67	1.95	1.77	1.45	1.67	1.81	1.64	1.65	1.73	2.02	1.80
Av. daily gain per dam, lb.	0.73	0.40	0.56	0.56	0.15	—.40	.01	—.09	—.18	—.51	—.54	—.41
Av. daily dry matter consumed per calf, lb.[c]	5.2	7.4	7.6	6.7	5.0	7.6	7.5	6.7	5.3	7.5	8.0	6.9
Estimated daily TDN consumed per calf, lb.				4.1				4.1				4.2
Calves fed forages and grain												
Av. initial calf age, days	118	138	147	134	108	148	143	133	118	145	142	135
Av. initial weight, lb.	237	311	321	290	230	319	320	290	243	312	310	288
Av. final weight, lb.	435	518	515	489	408	521	547	492	437	499	517	484
Av. daily gain per calf, lb.[b]	2.02	2.27	2.13	2.14	1.82	2.21	2.49	2.17	1.99	2.05	2.27	2.10
Av. daily gain per dam, lb.	.73	.35	.34	.47	—.17	—.63	—.38	—.39	—.04	—.59	—.36	—.33
Av. daily dry matter consumed per calf, lb.[c]	6.9	9.4	9.2	8.5	6.4	9.6	10.2	8.7	7.5	8.3	9.7	8.5
Estimated daily TDN consumed per calf, lb.				6.0				6.2				6.0

[a] There were 12 dams and calves per factorial treatment in 1955 and 1956 and 10 dams and calves in 1957.
[b] Gains were significant at the 1% level for years and rations.
[c] Dry matter was signaficant at the 1% level for years and rations.

There was no difference in the amount of dry matter consumed by the calves when associated to the three dam rations (table 4). This is evidenced by the 3 years' average daily dry matter consumption of 6.7, 6.7, and 6.9 lb. per calf on the corn silage-hay ration and 8.5, 8.7, and 8.5 lb. per calf fed the corn silage-hay-grain ration as related to the 100%, 75%, and 50% dam rations, respectively. The interactions of the dam rations × calf rations for dry matter consumption were not significant during any year nor for the combined data for the 3 years.

The constituents in the calf rations were fed *ad libitum*. Calves fed the corn silage-hay ration obtained 40% and 60% of the daily dry matter from corn silage and hay, respectively. Calves fed the corn silage-hay-grain ration obtained 15%, 35%, and 50% of their daily dry matter from corn silage, hay, and grain, respectively.

The results of supplementing the dam's milk with (a) corn silage-hay, (b) corn silage-hay-grain, (c) grain, and (d) no supplement when dams were on a 100% ration for two winter seasons are given in table 5. Daily gains and dry matter consumed by the calves were very consistent for a given supplement during each of the two years, but daily gains and dry matter consumed differed between supplements. When calves were restricted to their dam's milk, they averaged 0.33 lb. gain per head daily for the two years. Creep feeding of corn silage and hay resulted in gains of 1.81 lb. daily as compared with 2.20 lb. and 2.21 lb. daily for corn silage-hay-grain and the grain mixture, respectively. The dry matter consumed from supplements per 100 lb. of liveweight of the calves also differed, averaging 1.87 lb., 2.23 lb., and 1.42 lb. daily per calf, respectively, for the corn silage-hay, corn silage-hay-grain and grain rations.

Discussion

The experiments during the winters of 1952 and 1953 show that rations made up of (1) grass silage alone, (2) hay alone, or (3) combinations of these forages, with or without corn silage, make very satisfactory winter rations without supplementing grain for dams with suckling calves born during the summer. The same conclusion may be true for the calves, but the calves were not fed separately; hence, the value of the rations are difficult to ascertain.

In the experiments during the winters of 1955, 1956, and 1957, when feeding the dams a ration of corn silage alfalfa orchardgrass hay and grain, there were no apparent ill effects by reducing the ration level to 10.0–10.5 lb. of TDN daily. Each year weight losses sustained during the winter months were regained by July. New-born calves were normal in weight and vigor, and conception by the dams was 90% during a breeding season of 75 days. Although the 100% ration was very satisfactory for yearling-bred first calf heifers, the 75% and 50% rations hindered proper growth of these

TABLE 5. THE DAILY GAINS AND CREEP FEED CONSUMED BY SUCKLING CALVES ON FOUR RATIONS WHEN ALL DAMS WERE FED ON A FULL RATION [a]

Items	Forages			Forages+Grain			Grain			Dam's milk		
	1956	1957	Av.	1956	1957	Av.	1956	1957	Av.	1956	1957	Av.
Av. initial age, days	142	146	144	140	149	145	144	138	141	145	147	146
Av. initial weight, lb.	311	326	319	311	321	316	292	310	301	293	290	292
Av. final weight, lb.	463	503	483	518	515	517	483	520	501	327	313	320
Av. daily gain per calf, lb.[b]	1.68	1.94	1.81	2.27	2.13	2.20	2.10	2.32	2.21	0.38	0.27	0.33
Av. daily gain per dam, lb.	0.40	0.56	0.48	0.35	0.34	0.35	0.52	0.55	0.34	0.17	0.53	0.35
Av. daily dry matter consumed per calf, lb.												
Corn silage	2.6	3.2	2.9	1.3	1.2	1.3	...	...	...	...	...	...
Hay	4.8	4.4	4.6	3.6	3.3	3.4	...	...	...	...	...	...
Grain	...	...	...	4.5	4.7	4.6	5.7	5.7	5.7	...	...	...
Total	7.4	7.6	7.5	9.4	9.2	9.3	5.7	5.7	5.7	...	...	...
Estimated TDN intake per calf, lb.												
Corn silage			1.9			.9			...			...
Hay			2.6			1.9			...			...
Grain			...			3.7			4.6			...
Total			4.5			6.5			4.6			...

[a] There were six dams and calves per ration and five in 1957.
[b] Daily gains were significant at the 1% level.

heifers during the winter months. The lower gains and heavier losses by the dams on all the rations in 1956 and 1957, as compared to 1955, were considered to be due to their better condition when the experiment started rather than lower feed quality.

Satisfactory daily gains by the calves during these experiments show that summer dropped calves can effectively utilize high quality silages and/or hay when 4 months of age. Unpublished data by Hammes *et al.* (1958) show that Angus calves about 4 to 7 months old gained 1.8 lb. daily when fed good forage without grain. Dry matter digestibility of alfalfa-orchardgrass hay or silage fed to similar 5-month-old Angus calves ranged from 61.3 to 67.3%.

The low daily gains of 0.33 lb. made by the calves receiving only their dams milk, were not offset by high gains made on pasture. These calves were still somewhat stunted at the end of the grazing season. Therefore, a supplement to the dams milk is necessary, even when the dam is full-fed.

Gains made from 1955 to 1957 by calves supplemented corn silage and hay were considered low in cost and very satisfactory. However, the weaning weight of these calves was 35 lb. less per head than the weight of calves supplemented grain as part or all of their ration during the winter. Also, 47% of the calves supplemented corn silage and hay graded down into the "good" feeder grade while none of the calves receiving grain graded less than choice. There were no digestive difficulties from feeding a grain mixture as the only supplement; hence, it may be practical to feed only grain when silage and hay costs are high.

Calves from these experiments were used on summer grazing experiments. When fed grain at a rate of 1% of liveweight during the grazing season and slaughtered as long yearlings, the advantage in weight from feeding grain in the winter was maintained and directly contributed to higher carcass weights and grades. The weight advantage and carcass quality from feeding grain in the winter was not evident in the carcass if grain was not fed during the grazing season.

Summary

Winter feeding experiments with summer calves and their dams were conducted for 5 years. During 1955, 1956, and 1957, feeding experiments were conducted to study the effects of feeding 23.9, 18.0, and 15.5 lb. of dry matter daily to dams when the suckling calves were fed two rations in a factorial experiment. The average gains of the dams during the 3 years were 0.52, —.24, and —.37 for the 23.9, 18.0, and 15.5 lb. dry matter rations, respectively. Limiting the ration of the dams did not influence gains of calves.

The kind of supplement provided for suckling (July to September) calves, during the winter, had a decided influence on liveweight gains.

Calves gained 1.81, 2.20, and 2.21 lb. per head daily when supplemented corn silage-alfalfa orchardgrass hay, corn silage-alfalfa orchardgrass hay-grain and grain alone, respectively. Calves that were restricted to their dam's milk gained only 0.33 lb. daily.

The 1952 and 1953 experiments indicate that alfalfa-orchardgrass hay and/or silage are satisfactory forages for dams and their suckling calves.

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SUPPLEMENTS TO SILAGE RATIONS FOR FATTENING YEARLING STEERS

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THE results of numerous digestion trials and laboratory tests with the artificial rumen have shown that a wide variety of natural feeds as well as the ash or water extract of certain of these, will stimulate the breakdown and digestion of cellulose by rumen microorganisms (Burroughs *et al.*, 1950a; 1950b; 1951; Bentley *et al.*, 1953). In accordance with these results, several complex supplements have been designed for beef cattle rations containing low-protein roughages; for example, Purdue Cattle Supplement A (Beeson and Perry, 1952). Certain of these complex supplements have not been compared to simple oil meals on a protein-equal basis. Thus, little is known as to the practical benefits in terms of rate and economy of gain which may be obtained from their use, particularly where better quality roughages are fed.

In an attempt to determine the value of certain complex supplements and feed additives relative to soybean meal, a series of steer fattening trials were conducted at the Ft. Reno station from 1953 to 1957. Thirteen special supplements were compared to soybean oil meal; their relative value was determined by the rate of gain, efficiency of feed conversion and carcass merit of the steers.

Experimental

The trials were started each year in late September or early October. The steers used were long-yearling Herefords, grading good-to-choice and in moderate flesh and condition at the start of each trial. They were obtained from a large commercial herd in trial 1, and from the Experiment Station herds in trials 2, 3 and 4. Three or more supplements were compared in each trial.

In all trials, initial and final weights were obtained following a 16-hour period off feed and water to reduce differences in fill. The cattle were divided into uniform groups each of 10 head (12 steers per group in trial 1) on the basis of shrunk weight, source, feeder grade and previous summer gain; the groups were then assigned to treatment at random. The cattle were fed in large dirt pens adjacent to an open shed for shelter. The rations were offered once daily, the protein supplements and grain being poured over the silage in deep bunks. The trials averaged 166 days in length. At the completion of each trial, slaughter data and carcass grades were obtained.

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[2] Published with the approval of the Director.

Except in trial 1, a "two phase" feeding program was followed. Milo was limited to 4.0 lb per head daily for the first 84–100 days, followed by essentially a full-feed until the test was terminated. Silage was fed *ad lib.* so that the effect of the supplements on appetite could be observed. Immature corn silage was fed in trial 2 and Atlas sorghum silage in trials 1, 3 and 4. The only variation to this procedure was in trial 1, where milo was fed at the rate of 4.0 lb. per steer daily for the first 50 days, 8.0 lb. for the second 50-day period, and 12.0 lb. for the final 50 days of the 150-day test.

TABLE 1. AVERAGE PERCENTAGE CHEMICAL COMPOSITION OF SUPPLEMENTS AS FED

Supplement	Moisture	Ash	Crude protein	Ether extract	Crude fiber	N.F.E.	Ca	P
Soybean meal	9.42	6.26	46.63	1.18	5.83	30.68	.36	.56
Purdue supplement A[a]	9.35	12.17	33.71	1.95	7.63	35.19	1.85	.87
Supplement 3-1-1[b]	13.59	7.39	32.51	1.43	7.88	37.20	.56	.42
Supplement 1-1-1[c]	16.79	8.33	22.67	1.17	10.30	40.74	.62	.36
Special supplement[d]	7.35	9.30	28.55	2.76	10.39	41.66	.62	.56
S. B. meal—sesame mix[e]	9.74	8.52	47.09	3.18	5.82	25.65	.31	.83
S. B. meal—fermentation solubles[f]	8.14	8.50	41.92	2.35	5.99	33.10	.87	.77
S. B. meal—Aureomycin[g]	9.23	7.30	44.59	2.52	4.21	32.15	.34	.75
Vitamin-mineral mix[h]	8.95	8.29	35.99	1.00	5.74	40.13	1.57	.25
Urea-molasses mix[i]	29.73	8.33	30.60	....	.....	.....	....	...

[a] Soybean meal, 65.05%; molasses, 14%; dehydrated alfalfa meal, 14%; bone meal, 5.2%; salt (with 1 oz. $CoSO_4$/100 lb.) 1.7%; and vitamin A Source (10,000 U.S.P. units/gm.), 0.05%.

[b] Soybean meal, 3 parts; chooped alfalfa hay, 1 part, and blackstrap molasses, 1 part.

[c] Equal parts of soybean meal, chopped alfalfa and molasses.

[d] Dehydrated alfalfa meal, 35.0%; dried molasses, 35.0%; condensed fish solubles, 26.5%; dry live-cell yeast (20 bil. cells/gm.), 2.5%; and trace mineral mix supplying 100 mg. iron, 10 mg. copper and 2 mg. cobalt, 1.0%.

[e] Soybean meal, 67%, and sesame meal, 33%.

[f] Soybean meal, 78%, and dried grain and cane syrup fermentation solubles, 22%.

[g] Soybean meal, 97.5%, and crude chlortetracycline product (90 mg. Aureomycin per steer daily), 2.5%.

[h] Dried molasses, 0.79%; B-complex supplement (Fortafeed), 0.05%; dry stabilized vitamin A (30,000 U.S.P. units per gm.), 0.066%; ground limestone, 0.1%, and a commercial trace mineral mixture, 1 gm. per steer daily.

[i] Blackstrap molasses, 89.5%, and urea (Two-sixty-two compound), 10.5%. In addition the steers received 0.1 lb. steamed bone meal and 1 gm. commercial trace mineral mix per steer daily in concentrates fed.

The control group in each trial received 2.0 lb. soybean meal per head daily. Other supplements were fed on a protein-equal basis relative to the soybean meal. Where the supplements under test supplied more energy than the soybean meal fed to the control lot, adjustments were made in the milo allowance to provide equal energy intake from the concentrates fed. A mineral mixture of 2 parts salt and 1 part steamed bone meal was available to cattle in all lots.

The supplements tested and their chemical composition are shown in table 1. Ingredients used in the formulation of these supplements are shown in footnotes to this table. Certain of these supplements were recommended by other stations; others contained ingredients believed to be beneficial to rumen microorganisms. Where stilbestrol was fed, a premix containing the

desired amount was substituted for an equal amount of concentrates and mixed in a large vertical mixer in 30-day batches. A similar procedure was used in incorporating the crude chlortetracycline (Aureomycin) product in the supplemental mixture. Where Purdue Supplement A was fed, the amount of supplement needed during the trial was mixed at a commercial feed plant. Other supplements were mixed either daily (as in the case of the urea-molasses mixture) or in 30-day batches. Molasses was not mixed with other ingredients in Supplements 3–1–1 and 1–1–1, but was poured over the silage at each feeding [3].

Results and Discussion

A summary of average weight gains, daily rations, feed conversion and carcass data for trials 1, 2 and 3 is shown in table 2. Within this table, the various comparisons of supplemental mixtures to soybean meal have been grouped according to the trials in which they were fed. Where two trials were conducted, the values shown represent an average of the results obtained. Data for trial 4 are presented separately in table 3 since all cattle received 10 mg. stilbestrol in the daily ration.

It is apparent from the data that, with the exception of stilbestrol, none of the supplements tested was superior to soybean meal alone when fed on an equal-protein basis. These mixtures, ranging from relatively simple combinations of soybean meal, alfalfa and molasses (Supplements 3–1–1 and 1–1–1) to complex formulas such as Purdue Supplement A or the Special Supplement, were equally ineffective in promoting greater steer performance under the feedlot conditions encountered in this experiment. Further, none of the supplements had a marked effect on appetite, as indicated by silage consumption. Little difference is apparent in the milo or silage required to produce 100 lb. of gain as affected by the supplements fed, with the exception of those containing stilbestrol. Certain of the supplements, such as 3–1–1 and 1–1–1, gave more efficient gains in terms of milo required, but this was offset by greater quantities of supplement required per 100-lb. gain.

Dressing percentage and carcass grades among the lots were not significantly affected by the rations fed. There was a non-significant increase in dressing percentage and carcass grade where aureomycin was added to the ration. Other workers have noted a trend in the same direction. (Smith *et al.*, 1956).

The presence of alfalfa or molasses in the Purdue Supplement A, 3–1–1, 1–1–1, or Special Supplement gave no apparent improvement in steer performance. Beeson and Perry (1952) noted that the inclusion of dehydrated alfalfa meal in the Purdue Supplement A formula improved gains and feed

[3] Grateful acknowledgement is made to Standard Brands, Inc., New York, for the yeast; Calcium Carbonate Co., Chicago, for the commercial trace minerals; Eli Lilly and Co., Indianapolis, for stilbestrol and thyroid inhibitor; American Cyanamid Corporation (Lederle division), New York, for Aureomycin, and Texas Sesame Seed Producers, Paris, Texas, for the sesame meal.

TABLE 2. COMPARISON OF VARIOUS SUPPLEMENTS TO SOYBEAN MEAL FOR FATTENING YEARLING STEERS [a]

Supplements compared [b]	Trial no.	Av. daily ration (lb.)			Av. daily gain (lb.)	Feed per cwt. gain (lb.)			Dressing percent	Carcass grade score [c]
		Milo	Supplement	Silage		Milo	Supplement	Silage		
Soybean meal vs. Purdue A and Supple. 3-1-1										
Soybean meal	1,2	8.95	2.00	44.0	1.96	456	103	2248	59.9	6.2
Purdue Supplement A	1,2	8.68	2.77	42.4	1.85	468	150	2287	59.2	6.4
Supplement 3-1-1	1,2	8.49	2.93	42.2	1.99	426	147	2116	60.3	5.6
Soybean meal vs. Supplement 1-1-1 and special supplement										
Soybean meal	2,3	10.82	2.00	46.1	2.05	529	98	2251	60.7	5.6
Supplement 1-1-1	2,3	9.74	4.05	46.1	1.99	489	204	2316	60.3	5.7
S. B. meal-special supplement [d]	2,3	10.63	2.55	47.4	1.98	537	129	2399	61.0	5.5
Soybean meal vs. Soybean-sesame mix										
Soybean meal	2	9.84	2.00	47.6	2.09	471	96	2279	60.6	5.6
S. B. meal-sesame mix	2	9.88	2.02	47.9	1.99	497	102	2409	61.1	5.6
Soybean meal with various additives										
Soybean meal	3	11.80	2.00	44.6	2.01	588	100	2223	60.8	5.7
S. B. meal+fermentation solubles	3	11.60	2.25	47.4	1.98	583	114	2392	60.2	5.5
S. B. meal+aureomycin	3	11.80	2.00	43.5	2.15	548	93	2021	61.1	5.0
S. B. meal+stilbestrol [e]	3	11.80	2.00	49.5	2.50**	471	80	1975	60.5	5.2
S. B. meal+stilbestrol (last half)	3	11.80	2.00	47.1	2.33**	507	86	2022	60.1	5.7

[a] There were 12 steers per lot in trial 1 and 10 per lot in trials 2 and 3. Average initial weight was 683, 740 and 878 lb. for trials 1, 2, and 3, respectively.
[b] For composition of supplements see table 1.
[c] Carcass grade score based on choice=4, good=7 and standard=10.
[d] Soybean meal, 1.55 lb. special supplement, 1.0 lb. fed per steer daily.
[e] Stilbestrol, 10 mg. per head daily.
** Increased gain over other lots in this trial significant at the 1% level.

TABLE 3. COMPARISON OF VARIOUS SUPPLEMENTS TO SOYBEAN MEAL IN FATTENING RATIONS CONTAINING STILBESTROL [a]

Supplements compared [b]	Av. daily ration (lb.)			Av. daily gain (lb.)	Feed per cwt. gain (lb.)			Dressing percent	Carcass grade score
	Milo	Supplement	Silage		Milo	Supplement	Silage		
Without Antibiotic									
Soybean meal	12.6	2.00	38.3	2.60	484	77	1472	59.6	5.8
S. B. meal+vit. min. mix [c]	11.9	3.00	38.8	2.68	444	112	1449	60.2	5.4
Urea-molasses [d]	12.6	3.35	38.6	2.33	541	140	1658	59.7	5.4
S. B. meal+thyroid inhibitor [e]	12.6	2.00	39.0	2.59	486	77	1506	59.6	5.3
With Antibiotic [f]									
Soybean meal	12.6	2.00	40.2	2.69	469	76	1492	60.0	5.9
S. B. meal+vit. min. mix	11.9	3.00	37.3	2.57	463	119	1452	60.5	5.0
Urea-molasses	12.7	3.35	38.0	2.43	522	136	1563	60.6	5.2

[a] Trial 4. There were 10 steers per lot with the exception that one steer was removed from the lot fed soybean meal plus Aureomycin. Av. initial weight, 790 lb.

[b] For composition of supplements see footnotes to table 1. All cattle received 10 mg. stilbestrol in the concentrates fed.

[c] Two lb. soybean meal+1.0 lb. vitamin-mineral mixture.

[d] Urea-molasses mix replaced soybean meal by step-wise substitution during the first 21 days of the trial.

[e] Thyroid inhibitor (1-methyl-2-mercaptoimidazole) was fed at the rate of 150 mg. per steer daily during the last 99 days (finishing phase) of the trial.

[f] Aureomycin added in the form of the crude "Aurofac" product to supply 90 mg. activity per steer daily.

efficiency where corn cobs were the roughage. Richardson *et al.* (1953) observed an increase in gain when dehydrated alfalfa meal was added to a wheat straw ration for beef calves. Klosterman *et al.* (1953) found that the mineral fraction of either alfalfa or molasses would improve steer gains where corn cobs and weathered timothy hay were the roughages fed. The quality of the roughage may be the key to the improvement to be gained when molasses or alfalfa are included in supplemental mixtures. In the trials reported herein, the silage appears to have been a sufficient source of nutritive factors for the rumen bacteria, once its protein deficiency was overcome. Tillman *et al.* (1954a) found no improvement when alfalfa ash was added to rations containing prairie hay for sheep, but did obtain a beneficial response when it was added to rations containing cottonseed hulls (Tillman *et al.*, 1954b). Chappel *et al.* (1952) and Swift *et al.* (1951) observed an improvement in ration digestibility when alfalfa ash was added to corn cob rations for lambs.

Other additives also in the complex supplements appeared to be ineffective in promoting greater steer performance. Condensed fish solubles, live-cell yeast, B-complex vitamins, bone meal, ground limestone and trace minerals were included in the supplements tested with no apparent response. Apparently the silage was an ample source of carotene, since inclusion of vitamin A in several formulas was without effect. Dried grain and cane syrup fermentation solubles appeared to contain no additional nutritive factors. An attempt to improve the quality of protein in the supplement through a combination of 2 parts soybean meal and 1 part sesame meal (Almquist *et al.*, 1944) was of no benefit in trial 2.

Feeding 10 mg. stilbestrol in the soybean meal supplement in trial 3, significantly increased gains ($P < 0.01$) over the basal. Increases of 20% in rate of gain and 16.8% in feed efficiency (milo) were observed, which is in agreement with the results of Burroughs *et al.* (1955) and numerous other workers. In this trial, feeding stilbestrol throughout the fattening period resulted in greater gains than feeding it during only the last half (finishing phase), although differences between the two groups were not significant. This same tendency for greater performance when stilbestrol is fed throughout has been observed in trials with steer calves (Pope *et al.*, 1956).

The urea-molasses mixture fed in trial 4 (table 3) replaced soybean meal on a protein-equal basis. This resulted in slower and less efficient gains, although the differences were not statistically significant. Similar results have been obtained at this station (Gallup *et al.*, 1953) when pellets containing 85% of their nitrogen as urea were substituted for cottonseed meal. The use of a thyroid inhibitor with stilbestrol during the finishing phase of trial 4, as shown in table 3, failed to increase gain or improve carcass merit. Similar results have been obtained with steer calves fed the same compound (Pope *et al.*, 1957). Thus, the possibility of combining the beneficial effects of two drugs administered simultaneously, one to in-

crease gain and the other to improve fat deposition in the carcass, has not been demonstrated here.

The possibility of finding, in natural feeds or ration additives, growth factors stimulatory to rumen function and feed utilization proved disappointing under the conditions of this experiment. From this it appears that the basal ration composed of ground milo, soybean meal, silage and minerals (free choice) was adequate to meet the needs of rumen microorganisms as reflected by the weight gains of the steers. In general, these results have been consistent with those reportd by Smith *et al.* (1953), Richard (1954), Van Arsdell *et al.* (1953) and Duitsman and Kessler (1956).

Summary

Fattening trials involving 235 long-yearling Hereford steers were conducted to compare the value of certain simple and complex supplements to soybean meal in rations based on milo and either corn or sorghum silage. The relative value of 13 supplemental mixtures, when fed on an equal protein basis, was investigated. No consistent beneficial effects were obtained with any of the mixtures except those containing stilbestrol. Ingredients used in the supplements included alfalfa hay, dehydrated alfalfa meal, molasses, urea, condensed fish solubles, fermentation solubles, live-cell yeast, B-complex vitamins, vitamin A, steamed bone meal, trace minerals, Aureomycin, and a thyroid inhibitor. It would appear from the results that the basal ration containing milo, soybean meal, silage and minerals (free choice) was adequate to meet the needs of the rumen bacteria as measured by steer performance.

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THE EFFECTS OF TWO LEVELS OF ENERGY AND SEASONS ON REPRODUCTIVE PHENOMENA OF GILTS

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THE practice of over-feeding breeding animals has long been thought to be detrimental to maximum reproductive efficiency. For this reason many breeders limit the feed intake of their gestating gilts and sows to prevent obesity. This practice of limited feeding is generally carried out without considering the requirements for individual nutrients. Considerable evidence has been obtained during the last decade which indicates that a nutritional regimen of full feeding augments ovulation but is not contributory to intrauterine survival (Christian and Nofziger, 1952; Fowler, 1954; Robertson *et al.*, 1951b; Self *et al.*, 1955); however, in all of these studies restricted feeding was practiced which limited the intake of all nutrients and may have produced subclinical deficiencies of the essential nutrients required for maximum development. It would seem probable that a self-fed ration could be devised which would prevent excessive obesity by lowering the level of a single nutrient, such as one providing energy, without reducing the intake of other nutrients and be favorable to both the rate of ovulation and the survival of the embryos.

Present farrowing seasons may or may not exhibit full propensity to maximum reproductive phenomena such as age at puberty, ovulation rate and intrauterine survival. Hammond (1925) noted that the first fertile coitus in rabbits occurred at 8.5 months for does born in March to April and decreased to 5.3 for does born in October to December. Upp and Thompson (1927) reported similar data from chickens in which they stated that birds hatched during the winter, summer and fall months matured sexually at an earlier age than those hatched during the spring season, and earlier sexual maturity was associated with higher egg production. Wiggins *et al.* (1950) reported that the spring-farrowed gilts did not obtain puberty as early as those farrowed in the fall. These investigators also studied the effect of farrowing dates on age at puberty of gilts within a specific farrowing season and concluded that gilts farrowed late attain puberty earlier than those farrowed prior to the midpoint of the farrowing season. Robertson *et al.* (1951a) reported data which are in agreement with those of Wiggins *et al.* (1950) stating that gilts which were born later in the farrowing season tended to reach puberty at an earlier age. However, limited data which led to conclusions contrary to the previous work were reported by Warnick *et al.* (1951). Wise and Robertson

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(1953) found that the date of birth and the farrowing season under Texas conditions were apparently not associated with age at puberty. Self *et al.* (1955) stated that no significant relationship existed between the date of birth, farrowing season and age at puberty.

Consequently, these experiments were made to determine the effects of seasons and a ration of low energy content on various reproductive phnomena in gilts including age and weight at puberty, ovulation rate, and intrauterine survival.

Experimental Procedure

This study was composed of approximately equal numbers of Duroc, Hampshire and Poland China gilts farrowed during two different seasons. Gilts in Study I were farrowed in the spring of 1955 and those in Study II

TABLE 1. COMPOSITION OF DIETS

Ingredients	Diet 1[a]	Diet 2[b]
	lb.	lb.
Milo	56.0	20.0
Peanut hulls	00.0	30.0
Shorts (wheat gray)	15.0	15.0
Oats	15.0	15.0
Alfalfa leaf meal	4.0	4.0
Meat scraps	4.0	7.0
Soybean oil meal	4.0	7.0
Minerals[c]	2.0	2.0
Therms of productive energy[d]	93.1	55.3

[a] Diet 1 was fed to all control gilts (Group 1) in Studies I and II.
[b] Diet 2 was fed to all treated gilts (Group 2) in Studies I and II.
[c] Mineral mix composed of 1.5 lb. of bone meal and 0.5 lb. salt.
[d] Calculated according to the methods of Fraps (1946).

were farrowed in the fall of 1955. All gilts were assigned to the two lots in each study according to breed, weight and litter. The gilts in Studies I and II weighed 41 and 46 lb., respectively, and had an average age of 91 and 101 days, respectively, when placed on test.

Gilts receiving diet 1, as shown in table 1, which was calculated to contain 93 therms of productive energy (Fraps, 1946) per 100 lb. of feed served as controls. All other gilts were fed a lower energy diet containing 55 therms of productive energy per 100 lb. of feed so that energy intake was restricted while other nutrients remained relatively constant. All groups were fed *ad libitum* in a dry-lot approximately one acre in size. Equal housing and watering facilities were provided in each lot.

All gilts in these studies were checked daily for visible signs of estrus by examination of the vulvas for increased size and vascularity until they reached 120 days of age. Once this age was attained all gilts were checked daily for estrus with a vasectomized boar to determine age at puberty.

The gilts were checked daily after attainment of puberty for signs of second and third estrus. The gilts in Studies I and II were mated once to each of two different boars on the first and second day of the second estrous period. A double mating was used to insure fertilization of the greatest number of ova possible and to reduce any variations which might exist in the fertility of the boars. The various boars were mated to the same number of gilts in each group.

The gilts were allowed to reach 40 days of gestation before slaughtering. Within 30 minutes after slaughtering the uterine horns were carefully cut open to obtain data on number, length and condition of the embryos. Gross observations were made for any evidence of atrophy or presence of abnormalities. The corpora lutea on the ovaries were counted in order to determine the number of ovulations occurring at second heat. The percentage of prenatal survival was determined by the number of corpora lutea represented by normal living embryos.

The warm carcasses were weighed and stored in the cooler overnight at a temperature of approximately 1° C. Carcass measurements of the backfat and carcass length were made 24 hours after slaughter. The average thickness of the backfat was calculated by averaging the measurements taken on the cut surface of the carcass at the first rib, last rib and last lumbar vertebra. Carcass length was measured from the anterior edge of the first rib to the anterior edge of the aitch bone (pubis).

The various factors affecting reproductive performance including age at puberty, weight at puberty, ovulation rates, number of normal live embryos and percent live embryos were analyzed statistically by analyses of variance according to the methods of Snedecor (1956). Gross coefficients of correlation were calculated between all factors studied.

Results and Discussion

In Study I four gilts from group 1 failed to provide complete data. One gilt was removed from the test before attainment of puberty because of symptoms exemplifying parakeratosis. A second gilt which never attained puberty had an infantile reproductive tract when slaughtered at 315 days of age. Two others failed to settle and returned to estrus 20 days after breeding. Both reproductive tracts appeared normal when these gilts were slaughtered during metestrum following third heat. In group 2 of this same study three gilts failed to provide all data required; two never attained puberty while the third had only one estrous period and failed to cycle. These three gilts were sacrificed at approximately 325 days of age and infantile tracts were found in the two that never attained puberty while only follicles were noted in the gilt that failed to cycle.

Age at Puberty. The over-all average puberal age for the gilts in these two studies was 210.6 days (table 2). The average age at puberty for

the four groups ranged from 203.9 to 227.2 days with a standard error of 1.95. The individual range in all gilts was from 174 days to 274 days. Even though puberal age was attained 22 days earlier in the low energy group in Study I this trend failed to develop in Study II. The average age in the second study for the high energy group was 203.9 as compared to the low energy group of 207.9. However, when combining both studies the low energy group reached puberty at an average age of 206.5 days as compared to 214 days for the high energy fed gilts. This trend in favor of the low energy fed gilts for earlier attainment of puberty was

TABLE 2. SUMMARY OF EFFECTS OF ENERGY ON REPRODUCTIVE PHENOMENA (STUDIES I AND II)

Treatment	Number of gilts	Age at puberty, days	Weight at puberty, lb.	Number of ovulations	Number of normal embryos	Percent live embryos[a]
Study I						
High energy ration	12	227.2	204.9	13.3	7.6	59.9
Low energy ration	13	205.2	164.8	12.8	9.6	76.2
Difference		22.0	40.1	0.5	−2.0	−16.3
Average		215.7	184.1	13.0	8.6	68.3
Study II						
High energy ration	14	203.9	169.9	11.8	6.5	55.9
Low energy ration	13	207.9	155.0	9.9	7.0	72.7
Difference		−4.0	14.9	1.9	−0.5	−16.8
Average		205.8	160.3	10.9	6.7	64.0
Average for high energy ration	26	214.7	186.1	12.5	7.0	57.7
Average for low energy ration	26	206.5	166.3	11.4	8.3	74.5
Difference		8.2	19.8	1.1	−1.3*	−16.8**
Over-all average		210.6	171.7	11.9	7.7	66.1

[a] Average of individual means for each gilt.
** Significant at the 1% level of probability.
* Significant at the 5% level of probability.

not statistically significant in the combined analysis. When each study was considered separately a highly significant difference was noted in attainment of puberty due to rations fed. These data suggest that an earlier puberal age can be produced by reducing energy intake, provided that nutrients which are known to be essential for proper development of immature animals are present in comparable quantity as those found in the full fed rations.

These data are not in agreement with Haines *et al.* (1955) but their restriction in energy intake was more severe than the levels utilized in the present study. It agrees with the suggestion of Self *et al.* (1955) that "fatness" of the animal as conditioned by level of feeding may affect the attainment of puberty, but indicates that the level of energy fed is the principal contributing factor.

Gross coefficients of correlation were calculated from the combined data of Studies I and II as shown in table 3. Age at puberty and the weight at puberty were found to be correlated (0.45) while age at puberty was not significantly correlated with ovulation or live embryos. A small coefficient of correlation of 0.25 was found between age at puberty and percentage of live embryos which approached significance.

Weight at Puberty. The average weight at puberty for all gilts was 171.7 lb. (table 2) with a range from 131 to 252 lb. The average of

TABLE 3. CORRELATION COEFFICIENTS OF VARIOUS FACTORS MEASURED IN THESE STUDIES

Characteristics measured	r
Age at puberty and weight at puberty	0.45**
Age at puberty and number of ovulations	0.05
Age at puberty and number of live embyros	0.17
Age at puberty and percent of live embryos	0.25
Weight at puberty and number of ovulations	0.36**
Weight at puberty and number of live embryos	0.02
Weight at puberty and percent of live embryos	−0.31*
Ovulation rates and number of live embryos	0.44**
Ovulation rates and percent of live embryos	−0.38**
Ovulation rates and back fat	0.34*
Ovulation rates and carcass length	0.26
Live embryos and percent of live embryos	0.64**
Live embryos and back fat	−0.06
Live embryos and carcass length	0.21
Percent of live embryos and back fat	−0.35*
Percent of live embryos and carcass length	−0.07
Percent of live embryos and carcass weight	−0.40**

** Significant at the 1% level of probability with an r of 0.35 required.
* Significant at the 5% level of probability with an r of 0.27 required.

the four groups ranged from 155 to 204 lb. with a standard error of 3.16. As would be expected the gilts on the high energy ration in both groups tended to be heavier at puberty. The high energy gilts had an average weight of 186.1 lb. at puberty while the low energy gilts averaged only 166.3 lb. When each study was considered separately a highly significant difference was noted in weight at puberty due to rations fed. The combined analysis did not yield statistical significance, but the trend was the same in both studies, therefore these authors feel that the difference is real.

Weight at puberty was significantly correlated with ovulations (0.36) but was not correlated with number of live embryos. A negative correlation of –0.31 was noted for weight at puberty and percent live embryos. This indicated that full fed gilts which weigh more at puberty tend to have greater embryonic mortality.

Ovulation Rates. The average number of ova shed per gilt in the two

studies was 11.9 (table 2) with a range of 7 to 21. The means for the four treatment groups ranged from 9.9 to 13.3 with a standard error of 0.45. The high energy gilts in both studies showed a trend toward greater ovulation rates but this difference was not significant. When both studies were combined to evaluate the effects of rations, the high energy fed gilts ovulated an average of 12.5 ova as compared to 11.4 for the low energy fed groups. This difference of 1.1 ova does not approach statistical significance and is of interest since other workers (Robertson *et al.*, 1951b; Burger, 1952; Christian and Nofziger, 1952; Fowler, 1954; and Self *et al.*, 1955) have shown that a full feeding regimen is favorable to higher ovulation rates; therefore, it is concluded that the reduction in ovulation rates in limited fed gilts is not due to the lower energy intake but is due to the restriction of other nutrients in the ration.

Ovulation rates were highly significantly correlated with the number of live embryos (0.44) whereas they were negatively correlated (–0.38) with percent of live embryos. This is due to the fact that the number of live embryos tended to remain rather constant regardless of the number of eggs ovulated.

Number of Normal Embryos. An average of 7.7 embryos was recovered from the 52 gilts in these investigations. The range of embryos from individual gilts was 0 to 14 while the means of the four treatment groups ranged from 6.5 to 9.6 with a standard error of 0.35. The gilts consuming the low energy ration in both studies produced a larger number of normal embryos when slaughtered at 40 days of age than did the gilts receiving the high energy ration. When both studies were combined an average advantage of 1.3 embryos was noted for the low energy group. This was statistically significant at the 5% level of probability. The results of this study indicate that a greater number of 40-day-old embryos was present in gilts fed restricted energy diets. These results, that limited energy intake increases the average number of live pigs farrowed, are in agreement with the findings of Hanson *et al.* (1956).

The number of live embryos was found to be highly correlated (0.64) with percent live embryos as would be anticipated. However, the number of live embryos was not significantly correlated with backfat measurements or live slaughter weights.

Percentage of Live Embryos. In Studies I and II an average of 66.1% of the corpora lutea was represented by 40-day-old living embryos (table 2). The range in individual gilts varied from 0 to 109%. The latter figure can be explained by the possibility of twinning since a greater number of 40-day live embryos was recovered than the number of corpora lutea present on the ovaries. The high energy fed gilts in both studies had a greater percentage of intrauterine mortality than the low energy fed gilts. The gilts on the low energy ration had 74.5% of the corpora lutea represented by living embryos, which was statistically significantly different at the 1% level of probability from the high energy groups that had

an average of 57.7% of the corpora lutea represented by living embryos. Percentage of live embryos was significantly negatively correlated with backfat (–0.34) and live weight at slaughter (–0.35).

Effect of Seasons. Twenty-seven fall-farrowed gilts and twenty-five spring-farrowed gilts from the same herd and of similiar breeding in Study II attained puberty at an average age of 205 and 215.7 days, respectively, as shown in table 4. Although this difference of 9.9 days was not significant the fall farrowed group tended to attain puberty earlier. These findings are in accord with the work of Wiggins *et al.* (1950), since he noted that sexual immaturity was greatest in spring-farrowed gilts, but are not in agreement with Self *et al.* (1955).

TABLE 4. SUMMARY OF SEASONS ON REPRODUCTION PHENOMENA

Items	Number of gilts	Age at puberty, days	Weight at puberty, lb.	Number of ovulations	Number of normal embryos	Percent live embryos[a]
Spring farrowed	25	215.7	184.1	13.0	8.6	68.3
Fall farrowed	27	205.8	160.3	10.9	6.7	64.0
Difference	2	9.9	23.8	2.1*	1.9**	4.3

[a] Average of individual means for each gilt.
** Significant at the 1% level of probability.
* Significant at the 5% level of probability.

The same trend was noted for weight at puberty and for age at puberty since the fall-farrowed gilts averaged 160.3 lb. at puberty as compared to 184.1 for the spring-farrowed gilts. This difference of 23.8 lb. was not statistically significant.

The spring-farrowed group ovulated an average of 13.0 ova while the fall-farrowed gilts ovulated only 10.9. This difference of 2.1 ova in favor of the spring-farrowed group is statistically significant at the 5% level of probability. A highly significant difference of 1.9 embryos was noted in favor of the spring-farrowed gilts.

Summary and Conclusions

Fifty-two gilts were used to test the effects of seasons and two levels of energy in self-fed diets on the reproductive performance of gilts.

These studies were conducted to compare a control ration containing 93 therms of productive energy per 100 lb. with a ration containing only 55 therms of productive energy per 100 lb. Under the conditions of these investigations the lower energy appears more desirable since gilts on this treatment had a greater number of normal 40-day living embryos and a greater percent of living embryos present. Gilts receiving this ration also tended to reach puberty earlier and ovulation rates were comparable to gilts receiving the control ration.

Data are presented which demonstrate that a greater propensity for earlier attainment of puberty, maximum ovulation rates and higher embryonic survival can be attained by reducing the energy content of rations fed to developing and gestating gilts, providing the level of feeds containing essential nutrients known to be necessary for optimum growth of immature animals is maintained.

Under the conditions of this study the spring-farrowed gilts appeared to be more efficient in the reproductive processes measured except attainment of puberty. Ovulations in the spring-farrowed group were 19% greater and the number of 40-day-old embryos was 28% greater in gilts farrowed during this season. While the difference is not significant statistically, gilts farrowed in the fall tended to attain puberty at an earlier age.

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A COMPARISON OF EMBRYO SURVIVAL IN GILTS SLAUGHTERED TWENTY-FIVE VERSUS FORTY DAYS AFTER BREEDING

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DURING the last decade considerable information has been published regarding embryonic mortality in swine occurring from ovulation to 25 days of gestation. Concurrent data involving ovulation rates were also collected by counting the number of corpora lutea. Thus, an estimate of intrauterine survival could be made at 25 days of gestation by comparing the number of ovulations with live embryos. An evaluation of the reproductive efficiency of breeding swine could be established at a rather early stage of pregnancy by assuming that the existing embryos at this stage of gestation would reach parturition.

Warwick (1928) reported the largest percentage of degenerate fetuses occur in swine at an estimated gestational age of 20 days but this same author presented data which indicated that there was, on the average, a greater decline in the number of fetuses between 20 and 30 days of gestation than in any other 10-day period of gestation. A range from 15% to 80% of normal embryos at 25 days of gestation has been reported by Warnick *et al.*, 1948; Robertson *et al.*, 1951; Squiers *et al.*, 1952; Self *et al.*, 1955; and Lasley, 1952.

Glasgow *et al.* (1951) reported that the metabolites of progesterone or closely related substances decrease in concentration in the urine near the 25th day of gestation and these metabolites are highly correlated with the percentage of embryos implanted and the number of pigs farrowed. These investigations suggest that the secretion of progesterone by the corpora lutea decreases somewhat at this stage of pregnancy and the placental membranes begin the secretion of progesterone and continue the secretion at a rather high level during the later stages of gestation. Considering the possibilities that this hormone level is decreased at 25 days of embryonic life and that implantation occurs near this stage of prenatal development, it seemed reasonable that added stress during this stage of gestation would probably increase embryonic mortality, and could produce considerable error in estimating intrauterine survival. Glasgow *et al.* (1951) also found an increase in progesterone and its metabolites at approximately 40 days of gestation.

After slaughter of several gilts on the 25th day of gestation it was noted that difficulty was frequently encountered in distinguishing between

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living, possibly resorbing, and otherwise abnormal embryos. The distinction was based upon visual observation of the heart beat and gross abnormalities.

This study was made to compare 25- and 40-day slaughter groups of gestating gilts to determine which stage of pregnancy is the most accurate for estimating intrauterine survival.

Experimental Procedures

Reproduction data from a total of 85 slaughtered gilts were included in these comparisons. Thirty-three gilts were slaughtered at 25 days of gestation and 52 at 40 days. In both of the slaughter stages approximately one half of the gilts were fed a high energy ration and the remaining gilts were fed a low energy ration. These rations and their effects on the

TABLE 1. A COMPARISON OF 25- AND 40-DAY REPRODUCTIVE DATA IN MEASURING INTRAUTERINE SURVIVAL IN GILTS

Energy level	Number of gilts	Number of ovulations	Number of normal embryos	Percent live embryos [a]
		25-day gilts		
High energy ration	16	13.0	9.3	71.4
Low energy ration	17	12.0	9.1	70.8
Average		12.5	9.2	71.1
		40-day gilts		
High energy ration	26	12.5	7.0	57.7
Low energy ration	26	11.4	8.3	74.5
Average		11.9	7.7	66.1
Difference in averages		0.6	1.5	5.0

[a] Average of individual means for each gilt.

various reproductive phenomena of gilts are published in a companion paper (Gossett and Sorensen, 1959). Within 30 minutes after slaughter the uterine horns were removed and carefully cut open to permit observation of the number, length and general condition of the embryos. Gross observations were made for evidence of atrophy or presence of abnormalities. An embryo was considered normal and living if the heart beat could be detected and the embryo appeared normal, or, in the absence of heart beat, if no gross abnormalities were observed. The corpora lutea present on the ovaries were counted in order to determine the number of ovulations.

Results and Discussion

Ovulation rates were comparable for both groups (table 1). The gilts slaughtered after 25 days of gestation ovulated an average of 12.5 ova

as compared to 11.9 for the gilts slaughtered after 40 days of gestation. Since ovulation rates in both studies were closely related, the number of normal embryos for these two stages of pregnancy studied should also have been approximately equal. However, the data revealed that there was a survival difference in the embryos of the two stages of pregnancy when the number of ovulations was compared to the number of normal embryos. This indicated that some intrauterine mortality occurred after

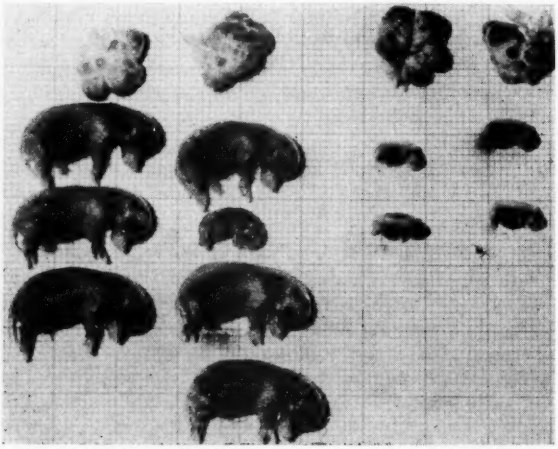

Figure 1. Twenty-five and forty-day-old embryos. This picture shows a comparison in size of embryos at 25 and 40 days of age. Ovaries and embryos are arranged according to their position in the reproductive tract. A resorbing 40-day-old embryo was removed from the right uterine horn. This embryo is larger than a normal 25-day embryo and likely would have been considered normal had the gilt been slaughtered at 25 days of gestation.

25 days of embryonic life. Specific reference is made to one particular gilt that was slaughtered at 40 days of pregnancy. The uterus of this gilt contained 5 resorbing fetuses that would have been considered normal at 25 days of gestation since the resorbing fetuses were larger than they would have been at that time. When comparing the percent of live embryos of the 25-day group with that of the 40-day group, it was observed that a decrease of 5.0 percentage points occurred during the extra 15 days of gestation.

An even more marked difference occurred in the high energy fed group slaughtered after 40 days of gestation as compared to 25 days of gesta-

tion. This difference was 13.7 percentage points. Therefore, the high energy group apparently had a higher mortality than did the low energy group after 25 days of gestation.

The difference in the size of embryos at the two stages of gestation studied is illustrated in figure 1 and points out the ease with which the

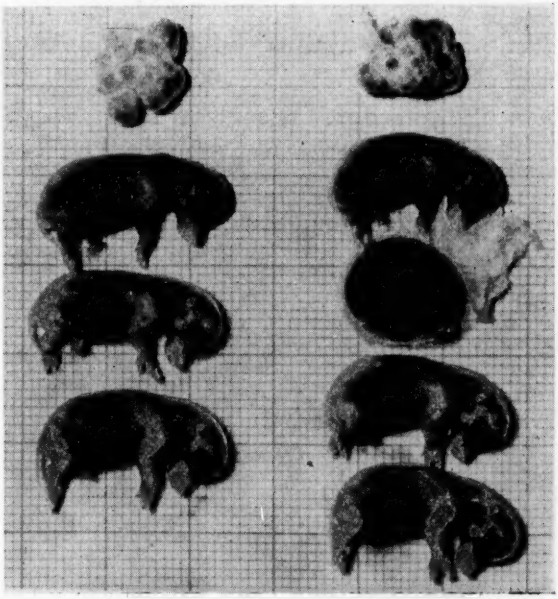

Figure 2. Forty-day-old embryos with one showing hemorrhaging. Hemorrhaging is shown in the second embryo in the right hand series. This embryo is abnormal but is larger than it would have been at 25 days. Therefere, it would have been considered normal at that stage of pregnancy introducing an error in evaluating intrauterine mortality.

abnormalities of the 40-day-old embryos can be detected. The crown-rump length of the 25- and 40-day embryos was approximately 18 and 49 millimeters, respectively. When slaughtering gilts at 25 days of gestation frequent hemorrhaging within the embryonic vesicles was observed. In such cases it was difficult to ascertain whether the embryos were normal or abnormal. It was unknown whether hemorrhaging occurred prior to or during the slaughtering process. A hemorrhaged condition readily detected by gross observation as being abnormal at 40 days of pregnancy

is shown in figure 2. Since this embryo is larger than a 25-day-old embryo, it probably would have been considered normal at that stage of gestation. A normal and a resorbing embryo at 40 days of age are shown in figure 3. The resorbing embryo is approximately 38 millimeters long which is 20

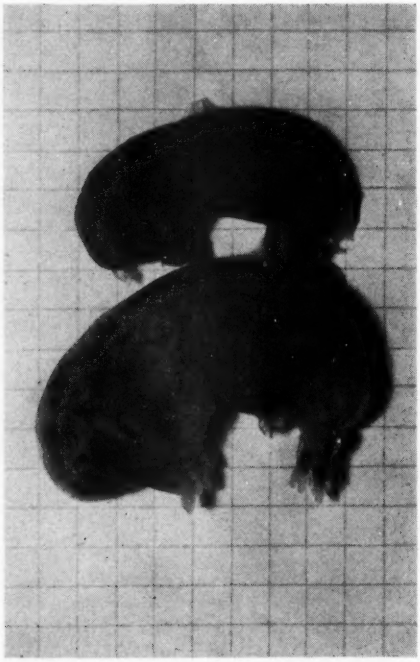

Figure 3. Normal and resorbing 40-day-old embryos. A normal 40-day-old embryo is contrasted with its litter mate which is shown to be resorbing but is approximately 10 millimeters longer than it would have been at 25 days of gestation.

millimeters longer than it would have been at 25 days of embryonic age, thus it would have been considered normal at 25 days of gestation. Figure 4 shows the intact placental membranes of a 25-day-old embryo and figure 5 shows the same for a 40-day embryo. The latter has more vascularity, an increased surface area and enlarged necrotic tissue. The increased area of attachment at 40 days is also evident.

Summary and Conclusions

Data were collected from 85 gilts to evaluate the difference in intra-uterine mortality at 25 and 40 day stages of pregnancy.

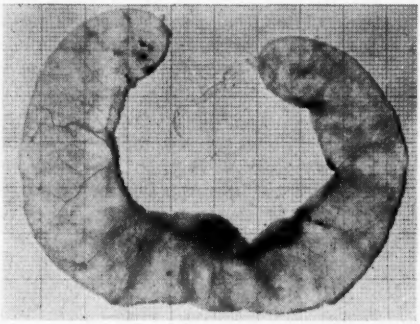

Figure 4. A normal 25-day placenta. The placenta of the 25-day-old embryo is strikingly different in surface area from the placenta of the 40-day-old embryo. Attachment is slightly diffused at this stage.

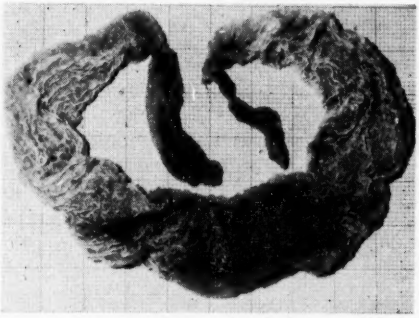

Figure 5. A normal 40-day placenta. A 40-day placenta shows an increase in surface area and the presence of necrotic tissue at the tips of the membranes, which is not evident in the 25-day placenta.

Under the conditions of these studies it appears preferable to slaughter gilts at 40 days of gestation to obtain an estimate of intra-uterine mortality. An additional 5 percentage points of mortality were found in the additional 15 days of pregnancy.

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CERTAIN SEMEN CHARACTERISTICS AND THEIR RELATION TO THE REPRODUCTIVE PERFORMANCE OF A PUREBRED HEREFORD HERD [1]

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AS the use of artificial insemination has increased, it has been necessary to develop methods to test the potential fertility of semen. A number of tests have been developed, but none of them is entirely accurate in predicting fertility. Buckner *et al.* (1954), Lasley and Bogart (1943), Lasley (1951), Erb *et al.* (1950), and Branton *et al.* (1951) used motility of semen to indicate potential fertility of semen. Ehlers and Erb (1950) found that the ability to maintain motility after storage was a better measure of fertility than initial motility. The correlations between volume per ejaculate and fertility have varied from high positive (Lasley and Bogart, 1943) to negative ones (Mercier and Salisbury, 1946).

Underbjerg *et al.* (1942) reported that stored semen, irrespective of treatment, showed a lower conception percentage as compared to fresh semen. Schultze *et al.* (1948) reported an average decline of 4.61% conception for each day of storage up to 4 days. Erb *et al.* (1950) reported a similar decline for 3 days.

Rather high dilution rates have been used without greatly lowering fertility (Willett, 1950; Salisbury *et al.*, 1943; Salisbury and Bratton, 1948).

Season is one of the important factors that has influenced the variation in semen quality and fertility. Phillips *et al.* (1943) reported semen concentration lowest in the fall, an increase of abnormal sperm in summer, and the lowest percentage of fertile matings in summer in Shorthorn and Milking Shorthorn cattle. Erb *et al.* (1942) and Schultze *et al.* (1948) observed a lowering of semen quality in summer. In areas of relatively mild summers and severe winters, semen quality has been reported to increase in summer and fall by Erb and Waldo (1952) and Mercier and Salisbury (1947).

Materials and Methods

The semen used in this study was collected from 31 Hereford bulls for the routine breeding operation of the Turner Hereford Ranch, Sulphur,

[1] The authors are indebted to Mr. Roy J. Turner, owner, and Mr. Jim McClelland, manager, of the Turner Ranch, Sulphur, Oklahoma for the data used in this study.

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Oklahoma, during the period from 1946 through 1952. There were 614 cows bred one or more times during the period covered by this study.

The herd was relatively free of disease during this time. A fulltime veterinarian was employed by Turner Ranch. No vibriosis or trichomoniasis has ever been diagnosed on the ranch. There were some cases of granular vaginitis. The semen was collected twice weekly by an artificial vagina. Only one ejaculate was taken in most instances. The volume, measured in ml., and initial semen motility ratings were made routinely. The motility ratings were 0 to 5, with 5 being the highest motility. Semen with a rating of 5 showed a very vigorous swirling movement that gave the appearance that nearly all sperm cells were in active motion. The lower ratings indicated a progressive decline in movement. Semen with a rating of 1 showed no mass movement whatsoever, only a few cells being in motion. The semen was diluted according to estimated needs. The greatest dilution used was 1:7. Until 1950 the semen was diluted in a half egg-yolk and half one-eighth molar phosphate buffer (Na_2HPO_4 and KH_2PO_4). The pH of the buffer was 7.2. After 1950 the diluter was half egg-yolk and half 3% sodium citrate, to which 1,000 units of penicillin and 1 mg. of streptomycin or dehydrostreptomycin per ml. of diluter was added. The diluted semen was stored at 36 to 38° F.

The cows were checked twice daily for heat. Any cows in heat were usually bred between 4 and 6 p. m., except for a few which were bred on the mornings that semen was collected. One ml. of semen was used per insemination.

The data were punched on IBM cards for ease of tabulation. The statistical methods used were those described by Snedecor (1946). The repeatability estimates of the number of services per conception were computed from components of variance as intra-class correlation.

Results and Discussion

Initial Motility. The average initial motility rating of semen used for 3,204 inseminations over the seven-year period was 4.85. Analysis of variance revealed no yearly differences in initial motility. Seasonal differences were significant at the 1% level. However, when examined for each season within each year, the seasonal changes in initial motility followed no definite pattern. Nevertheless, the initial motility tended to be lowest in summer and fall.

The analysis of variance indicated no difference in motility due to the age of the bull. However, there was a highly significant negative correlation of 0.62 between the age of the bull and semen motility. The correlation was determined from the average motility and the age of the bull. Also a significant negative regression of 0.018 was found for motility on age of bull. Deviation due to regression was significant at the 1% level. These figures indicate that as bulls get older they tend to produce semen

of lower motility. When the correlation was determined by age of bull within seasons it was reduced to a negative 0.22. This indicates that the true correlation was much nearer zero and that there is probably little difference in the motility of semen from bulls of different ages.

The relation of initial motility to fertility was determined by its effect on the number of services per conception. The average number of services per conception for each motility rating is shown in table 2. There were so few services from semen with motility below 2 that they were grouped in the class with a motility rating of 2. The chi-square value indicated

TABLE 1. CORRELATION AND REGRESSION COEFFICIENTS FOR SEMEN CHARACTERISTICS AND SERVICES PER CONCEPTION

Items	d.f.	r	95% Confidence limits	b	95% Confidence limits
Motility	2	—0.96*	—0.12 —0.99	—1.17*	—0.41 —1.93
Volume	6	0.42	1.32 —0.42	0.03	0.09 —0.03
Dilution	5	0.67	1.74 —0.22	0.14	0.29 —0.03
Age of semen at insemination	3	0.94*	0.99 0.30	0.47*	0.76 0.19
Age of bull	6	0.54	1.48 —0.28	0.06	0.14 —0.04
Age of cow	7	0.71*	0.93 0.05	0.12*	0.22 0.02

* ($P<0.05$).

that a deviation as large as this, or larger, would occur by chance less than 1% of the time. It is evident that as the semen motility increased the number of services per conception declined. This high relationship is evident from the significant negative correlation of 0.96 and regression of —1.17 (table 1). The deviations due to regression were also significantly greater than the deviations from regression, which means that the regression was significantly different from zero.

This seems to be somewhat in conflict with the fact that both motility and the number of services per conception tended to be lower in summer and fall. The correlation between motility and number of services per conception could still be high, since neither motility nor number of services per conception showed a definite relation to season when examined for each year. Also, as the correlation is based on initial motility and not on motility at the time of insemination, the fact that semen with low motility lost its fertility sooner than high-motility semen would tend to

TABLE 2. NUMBER OF SERVICES PER CONCEPTION AS AFFECTED BY INITIAL MOTILITY

Items	Motility rating				Total
	2 and below	3	4	5	
Total Services	22	103	357	2981	3463
Number of services per conception	5.50	5.15	3.00	2.32	2.42

Chi-square=39.2 3 d.f. ($P<0.01$).

cause the high correlation. That this did happen is shown in table 3. With semen that had a motility rating of 5, the conception rate was 1.84 services per conception on the day of collection and 3.58 services per conception on the third day after collection. The conception rate for semen with a motility of 4 was 1.94 and 10.75 services per conception on the day of collection and the third day after collection, respectively. Motility at the time of insemination would probably have been a better measure of fertility than initial motility.

However, a correlation as high as this indicates that initial motility in these data accurately measured semen fertility. This correlation is higher than most similar figures found in the literature. Lasley (1951) obtained a highly significant correlation of 0.31 between the percentage of motile spermatozoa and the conception rate in Hereford cattle. Lasley and Bogart (1943) found no relation between motility and fertility ($r=0.003$). In the present study the fertility of semen with motility scores of 4 and 3 was 23 and 65% less, respectively, than semen with a motility score of 5. Thus, with the cattle involved in this study, the use of semen with a motility less than 4 reduced the chances of conception to less than one in five.

Age of Semen and Fertility. The increase in the number of services per conception with increasing age of semen from the day of collection (zero) to 4 days or over is shown in table 4. There was a gradual increase in the number of services per conception up to 3 days and a slight drop

TABLE 3. NUMBER OF SERVICES PER CONCEPTION AS AFFECTED BY INITIAL MOTILITY AND SEMEN AGE

Age of semen (days)	Motility rating		
	3 and below	4	5
0	3.53 (53)[a]	1.94 (128)	1.84 (871)
1	10.00 (40)	3.05 (118)	2.30 (894)
2	15.00 (15)	6.18 (68)	2.61 (774)
3 and over	4.25 (17)	10.75 (43)	3.58 (441)

[a] Figures in parentheses indicate number of services.
Chi-square=9.8 ($P<0.14$) 6 d.f.

from 3 to 4 days. However, the sampling error with the small number of services in the 4-day group could account for this slight decline. The chi-square value showed that deviations as large as these, or larger, would occur by chance less than 1% of the time. The number of services per conception generally increased as the age of semen increased, regardless of the class into which the data were grouped.

The significant correlation and regression of the number of services per conception on the age of semen shown in table 1 also indicate that there is a relation between the two variables.

A decline in fertility with increased age of the semen has generally been observed. This decline has been approximately a 4% drop in conception rate with each day of age. The greatest decline occurred after the second day (Schultze *et al.*, 1948; Erb *et al.*, 1950; Swanson, 1951).

TABLE 4. NUMBER OF SERVICES PER CONCEPTION AS AFFECTED BY AGE OF SEMEN AT THE TIME OF INSEMINATION

Items	Age of semen (days)					
	0	1	2	3	4 and over	Total
Total number of services	1052	1052	857	469	32	3462
Number of services per conception	1.89	2.45	2.77	3.84	3.56	2.4

Chi-square=108.5 5 d.f. ($P<0.01$).

In this study there was a drop of 12.1% between the day of collection and the following day. Between the first and second day there was a 4.7% drop and a 10% drop from the second to the third day. In this study, fertility of the semen was reduced by about one-half by the third day.

Volume and Dilution. The volume per ejaculate was not significantly related to fertility as measured by the number of services per conception. This is evident from the correlation and regression in table 1.

The over-all averages indicate that semen volume tended to be lower in winter than in the other seasons. In 3 of the 7 years, semen volume was lowest in winter, and in two other years it was as low in winter as in any other season. In no year was volume highest in the winter.

The analysis of variance indicated no relation between the age of the bull and semen volume. The correlation, regression, and deviations due to regression of age of bull on semen volume were not significant. However, all these values approached significance, indicating that there was a tendency for the volume to increase with the age of the bull.

The correlation between dilution rate and services per conception and regression of services per conception on dilution rate shown in table 1 indicate that no relationship existed between fertility of semen and the dilution rates used in this study. This was not unexpected since much

higher dilutions have been used without materially affecting fertility (Salisbury *et al.*, 1943; Willet, 1950).

Additional Observations. The only relation between tests of semen quality possible in this study was between motility and volume. The correlation and regression were determined from the average volume and motility for the years involved. Neither the correlation of —0.26 nor the regression of —0.04 for motility on volume was significant.

The number of services per conception tended to be lower in summer and fall than in spring and winter. No significant seasonal difference in the number of services per conception was indicated by chi-square analysis. Further evidence of this is shown by the low negative correlation between the number of services per conception and average seasonal temperature. However, the season-within-year differences were significant ($P<0.01$), showing that the seasonal response was different from year to year. Thus there was no obvious seasonal trend in the number of services per conception, although when all services were grouped, summer and fall were lowest in the number of services per conception. There was no correlation between the seasonal rainfall and the number of services per conception and between yearly rainfall and the number of services per conception. There may be a tendency for cattle to be seasonal breeders, which could cause a decline in the number of services per conception in summer and fall. Apparently some factor(s) caused improved reproductive efficiency in summer and fall despite a decline in semen quality. The fertility of the cows might be raised enough by improved nutrition during the pasture season to more than offset the adverse effect of the lowered semen quality of the bulls.

The age of bull and age of cow differences in the number of services per conception were both significant, as shown by chi-square and by analysis of variance. There was no definite pattern between the number of services per conception and the age of the bull. However, the correlation between the age of the cow and the number of services per conception and the regression of the number of services per conception on age of cow were both significant. There was an indication that the relation might be curvilinear but the deviations from linearity were not significant. However, the number of services per conception decreased from 2 to 5 years of age and then increased above 5 years of age. Two-year-old cows required 2.45 services per conception and four- and five-year-old cows required 1.92 and 1.99 services per conception, respectively. Cows over 9 years of age required 3.23 services per conception. Generally heifers and old cows have required more services per conception than cows of intermediate age (Warren, 1958; Davis, 1951; Tanabe and Salisbury, 1946; Lasley and Bogart, 1943).

The most efficient matings were from five-year-old bulls mated to four-year-old cows. They required only 1.58 services per conception. On the average, the most efficient age groups were the four- and five-year-old

bulls mated to four- and five-year-old cows, with 1.74 services per conception. The least efficient groups were bulls over 5 years of age mated to cows over 6 years of age, with 2.91 services per conception. This is similar to the findings of Tanabe and Salisbury (1946), in which bulls 1 to 3 years of age mated to cows 4 to 6 years of age were the most efficient.

Repeatability of Success of Service. The repeatability of success of service was determined by an intra-class correlation utilizing the components of variance. The components yielded a repeatability of 0.08. With a repeatability as low as this, selection on the basis of early performance of a cow would be of little value. This repeatability agrees closely with values found by Carman (1955), Legates (1954), Pou *et al.* (1954), and Dunbar and Henderson (1953). The repeatability estimate of the number of services per conception in bulls in this study was 0.06.

Summary and Conclusions

The reproductive performance of the Turner Hereford Ranch Herd, Sulphur, Oklahoma, under artificial breeding for the period from 1946 through 1952 was studied. The motility of the semen was rated from 0 to 5 with 5 being the highest motility. The motility ratings averaged 4.85 for the 7 years. There were no important yearly differences in initial motility. The seasonal variation in initial motility was significant. Initial motility was lower in summer and fall than in winter and spring. There was an indication that as bulls get older, they produce semen of lower motility. The correlation between initial motility and the number of services per conception was —0.96, and the regression of the number of services per conception on initial motility was —1.17. Initial motility was not related to volume of semen per ejaculate.

The average volume of semen for the 7 years and for 31 bulls was 4.82 ml. The yearly and seasonal differences in volume were significant. Volume tended to be low in winter. There was a tendency for old bulls to produce a higher volume of semen than young bulls. The semen volume showed no relation to the number of services per conception.

There was no apparent relation between dilution rate and the number of services per conception. The age of the semen was closely related to the fertility of the semen. A significant correlation of 0.94 was found between the age of the semen in days and the number of services per conception. The significant regression of the number of services per conception on age of semen was 0.47.

Although the number of services per conception showed a tendency to decline in summer and fall, the difference was not significant. The rainfall by seasons was not related to the number of services per conception. The average seasonal temperature was not significantly related to the number of services per conception, though there was a tendency

for the number of services per conception to be low in summer and fall. Both age of bull and age of cow varied significantly in the number of services per conception. There was no consistent trend with age of bull. The significant correlation between age of cow and the number of services per conception was 0.71, and the significant regression was 0.12. There was an indication that younger and older cows were less efficient than cows of intermediate age. Cows 4 and 5 years of age mated to bulls 4 and 5 years of age were the most efficient. The least efficient matings were those of old cows and old bulls.

The cow repeatability of the number of services per conception was 0.08, determined by intra-class correlation without removing year and seasonal variance. The repeatability of bulls for number of services per conception was 0.06.

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STUDIES ON THE USE OF HORMONES IN LAMB FEEDING

I. Production Performance [1]

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THE administration of estrogenically active compounds to beef cattle and sheep during the growing-fattening period results in substantial increases in rate of gain and feeding efficiency (Andrews *et al.*, 1950; Jordan, 1950; Burroughs *et al.*, 1954; Hale *et al.*, 1955). These research findings have stimulated the use of such materials by commercial cattle feeders, but the practical application of such work to the sheep industry has been limited because of restrictions on the type and method of administration of these compounds to lambs. Some workers have shown that live and carcass grades of estrogen treated lambs are lower (Pope *et al.*, 1950; O'Mary *et al.*, 1952; Andrews and Beeson, 1953; Jordan, 1953). Reports of decreased carcass yields (O'Mary *et al.*, 1952) and increased amounts of moisture and offal (Whiting *et al.*, 1954) for hormone treated lambs indicate a reduction in the quality of carcasses from estrogen treated lambs.

The experiments reported here were conducted to investigate further the use of various estrogens for lamb fattening, both with respect to feedlot performance and to their effect on carcass quality.

Experimental Procedure

Trial I. One hundred and eight northwestern feeder lambs were divided into nine lots of 12 lambs each. Three different concentrate treatments were used; three lots received a ration based on shelled corn, three lots were fed cooked red kidney beans, and the remaining three lots received a ration of ⅓ shelled corn and ⅔ cooked red kidney beans, by weight. The kidney beans were prepared by cooking in canning factory retort for 15 minutes after a temperature of 240° F. was reached. Lambs in the corn-fed lots received 0.14 lb. of linseed meal per lamb daily and lambs on the other two treatments 0.10 lb. of linseed meal per day. All lambs were fed 0.50 lb. daily of clover hay and corn silage according to their appetite. Iodized salt was available in salt boxes.

Three hormone treatments were superimposed on these concentrate

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[2] The accompanying papers were part of a thesis submitted by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Animal Husbandry. Present address: Swine Research Branch, Animal Husbandry Research Division, Agricultural Research Service, U. S. Dept. of Agriculture, Beltsville, Md.

groups to give a 3 x 3 factorial design. Treatments were: (1) Stilbestrol fed at a level of 5.0 mg. per lamb daily for the first 58 days of the trial and 2.5 mg. for the remaining 33 days, (2) 100.0 mg. of progesterone and 10.0 mg. of estradiol [3] incorporated in a paste and implanted subcutaneously in the ear and (3) no hormone.

Hormones administered orally in all trials were incorporated into the linseed meal so that a feeding of 0.1 lb. of linseed meal per lamb daily would supply the hormone at the desired level. The lots implanted with progesterone-estradiol were given a second injection of the same amount at the end of the first 58 days on feed. The lambs were weighed individually at biweekly intervals in all trials and average daily feed consumption and feed consumed per hundred-weight gain were calculated. In all trials hormones given orally were withdrawn one week prior to slaughter.

Trial II. A 3 x 4 factorial design was used. Twelve lots of 12 northwestern feeder lambs were divided into the same concentrate groups used in Trial I. All lambs were fed 0.1 lb. linseed meal daily and corn silage, but received 0.5 lb. of alfalfa hay per day instead of the clover hay used in the first experiment. The hormone treatments used were: (1) Stilbestrol fed at a level of 5.0 mg. per lamb daily for the first 54 days and 1.0 mg. for the last 49 days, (2) 5.0 mg. of stilbestrol for the first 54 days and no hormone for the remainder of the trial,[4] (3) benzestrol [5] fed at levels of 5.0 and 1.0 mg. per lamb daily for 54 and 49 days, respectively, and (4) no treatment.

Trial III. Fifteen lots of 12 northwestern feeder lambs each were used in a 3 x 5 factorial experiment. The rations fed were the same as those used in Trial II except that the shelled corn—cooked bean concentrate was replaced by raw kidney beans. The hormone-treatment groups used in addition to a control group were: (1) Stilbestrol fed at levels of 5.0 mg. per lamb daily for the first 42 days and 2.5 mg. daily for the last 63 days of the trial, (2) stilbestrol fed as in (1) plus 0.75 gm. of thyroprotein for the duration of the trial, (3) stilbestrol as in (1) plus 0.50 gm. of thiouracil for the last 63 days of the experiment and (4) benzestrol fed at levels of 5.0 and 2.5 mg. per lamb daily for the 42- and 63-day periods, respectively.

Marketing and Slaughter

Similar procedures were followed in all trials. At the conclusion of the feeding period all lambs were graded by an experienced lamb grader. In Trials I and II, the lambs were shipped from the feeding barn by truck 28 miles to a rail shipping point. On off-loading they were weighed by hormone-treatment groups and then sent to New York City by rail,

[3] Furnished by Mattox and Moore, Indianapolis, Indiana.
[4] The designations stilbestrol (5–1) or stilbestrol (5–0) will be used in future references to these two treatment groups.
[5] Furnished by Hoffman-La Roche, Nutley, New Jersey.

a trip of about 30 hours. After arriving in New York they were given hay and water and again weighed by treatment groups. These weights were used to calculate shrink in transit. The lambs in these trials were slaughtered by treatment groups and their individual identity retained. In Trial III the lambs from the corn and cooked bean concentrate groups were trucked to Rochester, New York, for slaughter. Lambs in the raw bean group were not marketed at the end of the trial because of insufficient finish.

Prior to slaughter three lambs in each lot of twelve had been randomly selected as donors of nine-rib racks from which tissues for chemical analyses were obtained. These racks were removed as described by Hankins (1947). This gave a total of 27 racks in Trial I, 36 in Trial II, and 30 in Trial III.

TABLE 1. RESULTS OF EXPERIMENT 1

Item	Control			Stilbestrol (5–2.5)			Progesterone (100)--Estradiol (10)		
	Corn	1/3–2/3	Cooked beans	Corn	1/3–2/3	Cooked beans	Corn	1/3–2/3	Cooked beans
Lambs/lot	12	12	12	12	12	12	11	12	10
Av. initial wt., lb.	59.5	58.2	57.4	59.7	59.2	60.2	59.4	60.2	59.3
Av. final wt., lb.	89.4	87.9	88.3	96.6	96.4	98.2	92.6	96.8	96.7
Av. daily gain, lb.	0.33	0.33	0.34	0.41	0.41	0.42	0.36	0.40	0.38
Daily dry matter consumed, lb.	2.03	2.12	2.20	2.09	2.20	2.31	2.06	2.24	2.20
Dry matter/cwt. gain	620	647	616	517	535	559	566	555	583
Protein, % of D.M.	13.3	17.4	20.0	12.9	17.3	19.5	13.1	17.4	19.5

In Trial III the lambs selected were removed before marketing and slaughtered at the Cornell Meats Laboratory.

The carcasses were graded by a Federal grader. After slaughter hot carcass weights were obtained and used to calculate dressing percent.

The nine-rib racks from lambs in Trial I were physically separated into bone, fat and lean portions and chemical analyses was made on the fat and lean for moisture, protein, fat, and ash. In Trials II and III only the bone was separated and chemical analyses of the combined fat and lean were made.

Results and Discussion

Data on feed-lot performance are presented in tables 1, 2, and 3, and on carcass characteristics in tables 4, 5, and 6.

Rate of Gain. The average daily gain for all hormone-treatment groups in each trial was greater than that for control groups. In Trial I stilbestrol-fed and progesterone-estradiol implanted lambs gained on average of 0.41 and 0.38 lb. per lamb, respectively. These rates of gain were significantly higher ($P<0.01$) than that for control lambs (0.33 lb.). There were smaller differences in rate of gain for the second trial as the average daily

TABLE 2. RESULTS OF EXPERIMENT 2

Item	Control			Stilbestrol (5–1)			Stilbestrol (5–0)			Benzestrol (5–1)		
	Corn	1/3–2/3	Cooked beans	Corn	1/3–2/3	Cooked beans	Corn	1/3–2/3	Cooked beans	Corn	1/3–2/3	Cooked beans
Lambs/lot	10	11	12	11	11	12	11	12	12	12	12	12
Av. initial wt., lb.	55.8	56.8	57.0	56.7	55.4	55.8	56.7	56.8	55.9	56.1	56.0	55.3
Av. final wt., lb.	88.9	93.5	90.6	95.5	96.9	94.8	91.9	95.2	93.3	87.8	93.5	92.1
Av. daily gain, lb.	0.32	0.35	0.33	0.38	0.38	0.38	0.34	0.37	0.36	0.31	0.36	0.36
D.M. intake/day, lb.	2.15	2.34	2.34	2.11	2.24	2.36	2.03	2.30	2.31	2.11	2.29	2.26
D.M./cwt. gain	647	674	718	564	589	623	595	616	638	684	628	626
Protein, % of D.M.	13.5	18.4	20.9	13.3	18.3	20.8	13.3	18.3	21.2	13.3	18.3	21.2

TABLE 3. RESULTS EXPERIMENT 3

Items	Control			Stilbestrol (5–2.5)			Stilbestrol–Thyroprotein (0.75)			Stilbestrol–Thiouracil (0.50)			Benzestrol (5–2.5)		
	Corn	Raw beans	Cooked beans	Corn	Raw beans	Cooked beans	Corn	Raw beans	Cooked beans	Corn	Raw beans	Cooked beans	Corn	Raw beans	Cooked beans
Lambs/lot	11	12	12	11	12	12	11	12	11	12	12	11	10	12	12
Av. initial wt., lb.	61.5	56.9	63.3	60.2	56.2	62.0	58.8	53.5	58.3	60.7	57.7	60.5	61.0	56.8	61.7
Av. final wt., lb.	88.9	76.7	95.6	95.3	80.2	97.4	93.6	77.4	95.9	94.8	76.6	98.0	92.5	76.5	100.6
Av. daily gain, lb.	0.26	0.19	0.31	0.33	0.23	0.34	0.33	0.23	0.36	0.32	0.18	0.36	0.27	0.19	0.37
D.M. intake/day, lb.	1.90	1.77	2.33	1.99	1.74	2.33	2.18	1.83	2.31	2.11	1.71	2.26	1.91	1.87	2.46
D.M./cwt. gain	723	938	755	611	765	693	667	806	638	652	948	635	705	1000	664
Protein, % of D.M.	11.6	19.8	24.5	11.6	20.7	23.6	11.5	18.6	23.8	11.4	19.3	21.7	11.5	19.8	24.0

TABLE 4. MARKETING DATA

Treatment	No. of lambs	Shrink in marketing	Live grade: No. of lambs by grade				Live grade: Av.[1] grade	Carcass grade: No. of carcasses by grade				Carcass grade: Av.[1] grade	Hot dressing percent
Trial I			P	CH	G	U		P	CH	G	U		
Control	34	8.9	0	21	13	0	5.85	0	24	9	1	6.03	47.6
Stilbestrol (5–2.5)	36	6.6	0	12	18	6	4.50	0	13	20	3	4.83	46.0
Progesterone (100) Estradiol (10)	32	9.1	0	13	17	2	5.03	1	16	15	0	5.69	47.3
TRIAL II			CH	G	U			CH	G	U			
Control	32	10.4	7	18	7		4.00	3	25	3		4.00	44.9
Stilbestrol (5–1)	33	10.4	5	22	6		3.91	2	26	5		3.73	44.1
Stilbestrol (5–0)	35	11.1	5	21	9		3.66	1	27	7		3.49	44.5
Benzestrol (5–1)	36	9.7	5	27	4		4.08	3	27	5		3.83	44.1
Trial III			CH	G	U	CU		P	CH	G	U		
Control	23		8	14	1	0	4.91	6	10	1	0	7.88	
Stilbestrol (5–2.5)	22		8	8	5	1	4.23	1	6	8	0	5.60	
Stilbestrol–Thyroprotein (0.75)	22		4	11	7	0	3.59	0	8	3	4	4.80	
Stilbestrol–Thiouracil (0.50)	23		5	14	4	0	4.13	0	9	5	3	5.06	
Benzestrol (5–2.5)	22		11	9	2	0	5.23	5	10	1	0	7.75	

[1] Average grades were computed by assigning the following scores for each grade: Prime—10, Choice—7, Good—4, Utility—1, Cull—0.

TABLE 5. COMPOSITION OF NINE-RIB RACKS. TRIAL I

Treatment	No. of racks	Physically separated			Chemical composition							
					Fat				Lean			
						Dry matter				Dry matter		
		Fat	Lean	Bone	Moisture	Protein	Fat	Ash	Moisture	Protein	Fat	Ash
		%	%	%	%	%	%	%	%	%	%	%
Control	9	29.1	50.9	18.1	13.3	4.4	95.3	0.28	65.2	51.5	45.6	2.88
Progesterone (100) Estradiol (10)	9	27.4	52.1	18.7	14.6	6.0	93.7	0.28	65.7	51.3	45.9	2.78
Stilbestrol (5–2.5)	9	24.1	55.6	18.8	14.7	5.4	94.2	0.37	64.2	51.1	46.1	2.83

gain for treatment groups was controls, 0.33 lb.; stilbestrol (5–1), 0.38 lb.; stilbestrol (5–0), 0.36 lb.; and, benzestrol, 0.34 lb. These differences were not statistically significant. In Trial III all gains were lower but the trend remained the same as stilbestrol and stilbestrol-thyroprotein groups gained 0.30 and 0.31 lb. daily, vs. 0.25 lb. for the controls. These differences between supplemented and control lambs were significant. The stilbestrol-thiouracil and benzestrol-fed lambs gained 0.29 and 0.28 lb. per day, respectively, in this trial.

TABLE 6. COMPOSITION OF NINE-RIB RACKS. TRIALS II AND III

Treatment	No. of racks	Physically separated		Chemical composition of meat			
					Dry matter		
		Meat	Bone	Moisture	Protein	Fat	Ash
		%	%	%	%	%	%
Trial II							
Control	9	78.9	20.5	48.0	27.2	71.5	1.35
Stilbestrol (5–1)	9	76.8	22.2	55.3	34.6	63.8	1.74
Stilbestrol (5–0)	9	78.7	20.5	49.8	27.8	70.8	1.41
Benzestrol (5–1)	9	78.5	20.7	49.8	28.5	70.1	1.42
Trial III							
Control	6	71.2	28.6	45.3	23.0	75.8	1.17
Stilbestrol (5–2.5)	6	68.8	31.0	52.0	30.8	67.6	1.48
Stilbestrol-Thyroprotein	6	63.4	36.4	60.1	41.5	56.4	2.03
Stilbestrol-Thiouracil	6	68.2	31.7	52.4	31.4	67.0	1.50
Benzestrol (5–2.5)	6	66.7	33.0	50.0	27.7	70.8	1.41

The effect of the various concentrate treatments on daily gains was consistently in favor of the cooked bean treatments, although the differences were not large enough to be statistically significant in Trials I and II. In Trial I corn-fed lambs gained 0.37 lb. daily while lambs in the cooked bean and corn-cooked bean combination groups gained 0.38 lb. daily. When the same concentrates were used in Trial II, the average daily gains were 0.34 lb. for lambs receiving corn, 0.37 lb for the corn-bean group and 0.36 lb. for the all bean-fed lambs. In the third trial the differences among concentrate groups were more pronounced because of the substitution of uncooked kidney beans for the corn-cooked bean combination used in the previous trials. The use of raw kidney beans in lamb fattening rations had not proved satisfactory in earlier work as discussed by Johnson (1954). He attributed this lack of response to a decreased intake of raw beans by the lambs. Since hormone supplementation had been demonstrated, in some cases, to increase feed consumption, it was hoped that this hormone effect would result in a greater intake of the uncooked beans and in a corresponding increase in growth rate.

To a limited extent this took place, but as the average daily gains for concentrate groups in Trial III show (raw beans, 0.20; corn, 0.30, and cooked beans, 0.35 lb.), the overall result was very poor. Not only was rate of gain low, but feed consumption and efficiency decreased. The differences in rates of gain were highly significant for all three treatments. Other significant effects on gain in Trial III were due to differences in rate of gain between allotment groups (based on initial weights), and a significant interaction between allotment groups and concentrate treatments. The data indicated that in this trial, in contrast to the earlier ones in which no initial weight effect was noted, lighter lambs made slower gains than heavier ones and the effect was particularly noticeable for lambs receiving raw kidney beans. This suggests that factors other than decreased feed intake influenced the response of lambs to this concentrate.

Feed Consumption. The use of the various hormones and combinations in the three trials did not have a consistent effect on feed consumption. In Trials I and III there was a tendency toward increased feed consumption in hormone-treated lots but this did not occur in the second trial. In contrast to the slight effect of the hormones on feed intake, there were marked differences due to concentrate treatment. Lambs receiving rations containing cooked kidney beans uniformly consumed more feed, as the data on daily dry-matter intake given in the tables 1, 2 and 3 show. The inclusion of the raw kidney beans resulted in a much lower daily feed consumption which was only slightly increased by hormone supplementation. There was a wide spread in protein content between the various concentrate rations and this may have been a factor influencing the greater intake for lambs fed cooked beans and their somewhat faster gains.

The increased intake of feed in treatment groups getting cooked beans was accompanied by a slight decrease in efficiency of gain in all trials. In Trial III the raw-bean concentrate group showed a decreased feed consumption and also a marked decrease in feed efficiency. The hormone treatments uniformly increased feed efficiency in all trials. In each trial the stilbestrol-treatment group made the most economical gains. Inspection of the averages indicated that rapidity of gain and economy were closely related. In Trial III the addition of thyroprotein to stilbestrol reduced efficiency of gain slightly (690 vs. 704 lb. per cwt. gain) when compared with the lambs fed stilbestrol, but both of these values were noticeably better than that for the controls (805 lb. per cwt. gain). The addition of thiouracil to stilbestrol during the last half of the feeding period in one treatment group slowed the rate of gain, but also slightly decreased feed efficiency (745 lb.). On the other hand, the benzestrol-treatment group's feed consumption per hundred weight (790 lb.) was only slightly better than the consumption value obtained for the controls.

Feed-lot Losses. Losses thought to be caused by hormonal supplementation were relatively few. No ewe lambs were removed from test because of death or undesirable side effects due to the hormone use. In Trial I three wether lambs in the progesterone-estradiol treatment group either died or

were removed because of urinary blockage and in Trial III two wethers in the stilbestrol and stilbestrol-thyroprotein groups were lost for the same reason. Of a total of 432 lambs used in the three trials, 186 were wethers receiving one of the various hormone treatments. Of these only five were lost because of hormonal side-effects. Other losses were not attributed to experimental treatment.

Live and Carcass Grades. In table 4 the live and carcass grades for hormone treatment groups are given for all trials. In Trial I the control lambs were the most highly finished. Their average live grade of 5.85 was significantly higher than the 4.50 for stilbestrol-fed lambs, but was not significantly different from the average grade of progesterone-estradiol implanted lambs (5.03). Carcass grades for this trial were comparable to live grades and the difference between control (6.03) and stilbestrol-fed lambs (4.83) was significant.

In Trial II the differences between treatment groups for these factors were much smaller although the tendency for untreated lambs to grade higher was still apparent. One exception to this trend was noted however; the live grades for benzestrol-fed lambs were slightly higher than controls (4.08 vs. 4.00) and only slightly lower in the carcass (3.83 vs. 4.00). These smaller differences in grades for lambs in Trial II seem to reflect the smaller differences in rate of gain also found in this trial.

The grades in Trial III were in line with those obtained in previous trials. Again the effect of benzestrol found in Trial II was noted as live grades for these lambs averaged 5.23 while the controls graded 4.91. The carcass grades also were very close, 7.75 for benzestrol-fed lambs vs. 7.88 for controls. The higher carcass grades for Trial III do not reflect a greater degree of finish for the lambs in this trial, but rather a revision of Federal grading standards which resulted in an apparent upgrading for all lambs in Trial III.

Another finding of this trial was the failure of thiouracil addition to stilbestrol to raise the grades of lambs in that group. It is believed that the level of thiouracil (0.5 gm. per lamb daily) was too low to be effective. The addition of thyroprotein to stilbestrol produced a lamb with even less finish than did stilbestrol alone.

The consistent finding of lower live and carcass grades for hormone-treated lambs, with the exception of benzestrol-fed ones, did not result in a decreased return per lamb under the conditions of these trials. The greater feed efficiency with resulting lower feed costs generally resulted in a greater net return per lamb for those receiving the supplements.

Shrink and Dressing Percentage. Data for shrink in marketing obtained for hormone treatment groups in Trials I and II (table 4) fail to show any striking differences between treatments and give no indication of increased shrinkage for treated lambs. The data for dressing percent also do not point out any marked treatment effects. Since the data in Trial III were obtained by concentrate treatment groups they are not included in table 4,

but the differences in dressing percent were very slight although corn-fed lambs showed more shrink in transit than did bean-fed (5.38% vs. 3.25%).

Carcass Composition. Data on physical separation and chemical composition of the nine-rib racks from Trial I are given in table 5 and data for Trials II and III in table 6. The values for percent of bone in the racks show that the racks from control lambs in each trial had the lowest percentage of bone. The higher percentage of bone found for all treatments in Trial III indicates a less careful separation procedure and not a gross difference in composition of the racks. Separation data for Trial I also show a higher percent of separable fat in control racks, while chemical analysis shows a lower water content of this fat. No increase in moisture was found in the separable lean from treated lambs. Further inspection of the values for chemical composition indicates relatively small differences, only those for protein and ash in fat were statistically significant. The practical significance of these differences is probably negligible and may be due to variations in the precision with which the physical separation of the nine-rib racks was carried out.

The chemical analyses of the combined fat and lean in Trials II and III gave comparable results for each trial. The meat from control lamb racks had less moisture and the dry matter contained less protein and ash and more fat. In both Trials II and III stilbestrol treatment caused the most marked variation from controls and the thyroprotein-stilbestrol treatment in Trial III tended to exaggerate the stilbestrol effect. The combination of the data for chemical analyses of separated fat and lean in Trial I gives a composition picture very similar to that seen in Trials II and III.

Considering the various factors studied, the general effect of hormones on the carcass appears to be to produce a carcass more characteristic of an immature animal. The higher percentage of bone, moisture, protein and ash and lower fat content of racks from treated lambs indicates that growth is still an active process. Thus it may be necessary to feed estrogen-treated lambs to heavier weights before marketing in order to produce carcasses with the desired degree of finish.

Summary

In a series of three feeding trials with 432 lambs several estrogenically active compounds were studied to determine their value for the production of market lambs. In Trial I lambs which received 100 mg. progesterone—10 mg. estradiol implants or were fed 5 mg. of stilbestrol reduced to 2.5 mg. by the end of the feeding period gained significantly faster than control lambs.

In a second trial lambs fed stilbestrol either continuously or for the first half of the feeding period (levels of 5 mg. per lamb later reduced to 1 mg. or zero) gained slightly faster than controls. Benzestrol-fed lambs also showed some increase in rate of gain, but none of these increases was significant.

A third trial in which stilbestrol (5 mg. per lamb later reduced to 2.5 mg.) was fed either alone or in combination with thyroprotein (0.75 gm. per lamb daily) or thiouracil (0.50 gm. per lamb daily for the last half of the trial) again demonstrated significantly faster gains for both stilbestrol and stilbestrol-thyroprotein supplemented lots. Thiouracil addition decreased the rate of gain below that of these two groups as did benzestrol.

Estrogenic hormone use in all trials increased feed efficiency and the faster gaining lambs tended to make the most economical gains. In comparison of concentrate treatments containing either shelled yellow corn or cooked red kidney beans, the latter gave faster gains in all trials and the difference was significant in the third trial. The feeding of uncooked beans to a group on Trial III resulted in a reduced feed intake and significantly slower gains. The faster gains resulting from feeding the cooked kidney beans were not accompanied by an increased efficiency of gain.

All supplements used except benzestrol consistently produced lambs that graded lower, both alive and in the carcass, than did control lambs. They increased the moisture content of the edible tissue of the carcasses and also increased the protein and ash but lowered the fat content of the dry matter. The supplements which caused the greatest alteration in carcass composition were those that promoted the fastest growth rate, stilbestrol in all trials and the stilbestrol-thyroprotein combination in Trial III.

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STUDIES ON THE USE OF HORMONES IN LAMB FEEDING

II. Tissue Assays and Physiological Effects

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THE possibility of finding hormone residues in tissues from animals treated with estrogenic compounds must be considered in evaluating the results of a study involving these materials. The Food and Drug Administration of the U. S. Department of Health, Education and Welfare does not endorse the distribution of meat from hormone-treated animals if the meat contains any of the added hormone (Federal Register, 1948). Stob *et al.* (1954, 1956) detected significant estrogenic activity in the meat of steers and wethers implanted with synthetic estrogens, as well as in various tissues from cattle treated orally with three different estrogenic compounds. Ellis *et al.* (1954) showed that lambs implanted with diethylstilbestrol retained small amounts of estrogenic material in their tissues. Andrews *et al.* (1956) failed to detect any estrogenic activity in lean and fat of lambs treated orally with the synthetic estrogen, dienestrol, but found highly significant amounts of estrogen in their livers. The present study was undertaken to determine if any estrogenic residues remained in the edible portions of the carcasses of lambs fed two synthetic estrogens, diethylstilbestrol and benzestrol, and of lambs injected with an estradiol-progesterone combination, as well as to describe certain other physiological side effects which resulted from these hormone treatments.

Experimental Procedure

Estrogen bioassays were conducted on samples of tissues taken from 9-rib-racks of lambs representative of each of the hormone treatments in lamb feeding trials reported by Davey and Wellington (1958). In addition, liver samples from trial I were assayed. The hormone treatments are listed in table 1.

In all trials, feeding of hormones was stopped one week prior to slaughter. In trial I, lean and fat tissues were physically separated and samples from nine carcasses from each treatment group were dried under vacuum, pooled and equal amounts of dried tissue from each treatment group were extracted continuously for 48 hours with diethyl ether. The dried ether extracts were used for the assay. Similarly pooled liver samples were also obtained, dried under vacuum and equal amounts of dried tissue from each treatment group were extracted for 48 hours with ether. The dried

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ether extracts of the livers were subsequently extracted for an additional 48 hours with 95% ethanol in an effort to concentrate further any estrogenic materials. The ethanol extract was then used for the bioassay.

In trial II, no attempt to separate lean meat from fat was made. However, rib-rack tissue samples from bean-fed lambs were extracted and assayed separately from samples from corn-fed lambs. Equal amounts of dried tissue from pooled samples from nine lambs in each treatment group

TABLE 1. HORMONE TREATMENTS

Trial no.	Group no.	Hormone treatment
I	1	Untreated
	2	Stilbestrol-fed (5 mg./day for first 58 days, 2.5 mg./day for remaining 33 days)
	3	Progesterone-estradiol implanted (100 mg. progesterone and 10 mg. estradiol implanted subcutaneously in ear at beginning of experiment, and again 58 days later)
II	1	Untreated
	2	Stilbestrol-fed (5 mg./day for first 54 days, 1 mg./day for remaining 49 days)
	3	Stilbestrol-fed (5 mg./day for first 54 days, none for remaining 49 days)
	4	Benzestrol-fed (5 mg./day for first 54 days, 1 mg./day for remaining 49 days)
III	1	Untreated
	2	Stilbestrol-fed (5 mg./day for first 42 days, 2.5 mg./day for remaining 63 days)
	3	Stilbestrol+thyroprotein (stilbestrol same as group 2, plus 0.75 grams iodinated casein orally per day for duration of feeding period)
	4	Stilbestrol+thiouracil (stilbestrol same as group 2, plus 0.50 grams thiouracil orally per day for the last 63 days of feeding period)
	5	Benzestrol-fed (5 mg./day for first 42 days, 2.5 mg./day for remaining 63 days)

were extracted for 24 hours with n-butanol, the resulting extracts being used for assay purposes. Additional tissue samples from bean-fed lambs which had received hormone treatments for the duration of the experiment were extracted with ether to obtain a comparison of the efficiency of extraction of estrogenic residues by the two solvents.

In trial III, equal amounts of dried rib-rack tissue samples from the corn-fed lambs on each hormone treatment were extracted for 24 hours with diethyl ether and these extracts were used to conduct the bioassay after removal of the ether.

The bioassay procedure used was a modification of the 72-hour assay described by Lauson *et al.* (1939), based upon the increase in uterine weight of immature rats as a result of increasing doses of estrogen. At

least 10 rats, 21 to 23 days of age, were used in the assay of each tissue extract. The materials to be assayed were liquified by warming prior to injection, and each rat received one subcutaneous injection per day, for 3 days. Twenty-four hours after the last injection, the rats were weighed and sacrificed. The uteri were removed, dissected free of surrounding connective tissue, blotted on filter paper to remove excess moisture, and weighed immediately on a Roller-Smith torsion balance. In trial I, 0.1 ml. of extract was injected per rat per day, while in trials II and III, 0.2 ml. were injected daily into each rat.

TABLE 2. ESTROGENIC RESIDUES IN TISSUES, TRIAL I

Substance assayed	Lamb treatment	No. of rats	Mean adjusted uterine weight	Stilbestrol equivalent per kg. tissue
			mg.	mcg.
Liver extract	Untreated	11	16.56	
	Stilbestrol-fed (5 mg.–2.5 mg.)	11	17.82**††	1.7
	Progesterone-estradiol implanted (100 mg.–10 mg.)	11	17.42*†	2.8
Lean extract	Untreated	10	18.13††	20.8
	Stilbestrol-fed (5 mg.–2.5 mg.)	10	17.90††	22.1
	Progesterone-estradiol implanted (100 mg.–10 mg.)	11	19.12**††	24.3
Fat extract	Untreated	12	19.80††	54.4
	Stilbestrol-fed (5 mg.–2.5 mg.)	12	22.63**††	69.6
	Progesterone-estradiol implanted (100 mg.–10 mg.)	12	19.40††	52.4
Sesame oil		11	16.65	

* =Significantly ($P<0.05$) different from extracts of tissues from untreated lambs.
** =Significantly ($P<0.01$) different from extracts of tissues from untreated lambs.
† =Significantly ($P<0.05$) different from untreated rats.
†† =Significantly ($P<0.01$) different from untreated rats.

In addition to the tissue assays, two standard curves were established, one using four different levels of estradiol-17 beta, and the other using four different levels of stilbestrol. In each case the response to increasing dose was linear within the range of doses which was used (0.006 micrograms per day to 0.050 per day for estradiol; 0.005 micrograms per day to 0.040 per day for stilbestrol). In the assays of both the standards and the unknown, the uterine weights were adjusted by covariance for variations in body weight (Snedecor, 1956).

In order to assess certain physiological side effects of the hormone treatments, weights of the uteri, ovaries, seminal vesicles and bulbo-urethral glands were obtained from lambs selected for carcass composition studies reported in the accompanying paper (Davey and Wellington, 1958).

Additional endocrine glands saved in trial III were thyroids, adrenals and pituitaries. The thyroids and adrenals were fixed in Bouin's solution and sections made for histological examination.

Results and Discussion

Tissue Assays

The results of the tissue assays for trials I, II, and III are presented in tables 2, 3 and 4, respectively.

TABLE 3. ESTROGENIC RESIDUES IN TISSUES, TRIAL II

Substance assayed	Lamb treatment	No. of rats	Mean adjusted uterine weight	Stilbestrol equivalent per kg. tissue
			mg.	mcg.
Fat+lean, butanol extract	Untreated, corn-fed	10	17.57	
"	Untreated, bean-fed	10	17.11	
"	Stilbestrol, 5 mg.–1 mg., corn-fed	10	17.73	
"	Stilbestrol, 5 mg.–1 mg., bean-fed	10	18.27	
"	Stilbestrol, 5 mg.–0, corn-fed	10	17.63	
"	Stilbestrol, 5 mg.–0, bean-fed	10	18.44	
"	Benzestrol, 5 mg.–1 mg., corn-fed	10	17.61	
"	Benzestrol, 5 mg.–1 mg., bean-fed	10	18.88**†	16.5
Fat+lean, ether extract	Stilbestrol, 5 mg.–1 mg., bean-fed	10	18.12	
"	Benzestrol, 5 mg.–1 mg., bean-fed	10	19.98**††	20.6
Corn oil		10	18.10	

** =Significantly ($P<0.01$) different from extracts of tissues from untreated lambs.
† =Significantly ($P<0.05$) different from untreated rats.
†† =Significantly ($P<0.01$) different from untreated rats.

After adjusting uterine weights to a uniform body weight basis by covariance, the differences between mean adjusted uterine weights were tested for significance by analyses of variance and Duncan's multiple range test (Duncan, 1954). In trial I (table 2) extracts of livers from stilbestrol-fed and progesterone-estradiol injected lambs both contained significantly ($P<0.01$ and $P<0.05$, respectively) more estrogenic substances than extracts of livers from untreated lambs. The comparison of lean extracts indicated significantly ($P<0.01$) more estrogenic residue in muscle from progesterone-estradiol injected lambs than in untreated

lambs, while in the comparison of fat extracts, the extracts from stilbestrol-fed lambs contained significantly ($P<0.01$) more estrogenic residue than extracts from untreated lambs.

In addition to these findings, the lean and fat extracts from untreated lambs also contained detectable amounts of estrogenic materials as indicated by the increased uterine weights of rats injected with these extracts over those of rats receiving only injections of sesame oil. It is not known whether these tissue residues in untreated lambs arose from endogenous sources, or exogenously from some feed constituent.

TABLE 4. ESTROGENIC RESIDUES IN TISSUES, TRIAL III

Material assayed	Lamb treatment	No. of rats	Mean adjusted uterine weight	Stilbestrol equivalent per kg. tissue
			mg.	mcg.
Fat+lean, ether extract	Untreated	10	18.72	
"	Stilbestrol (5 mg.–2.5 mg.)	10	19.52	
"	Stilbestrol+thyroprotein (.75 gm.)	10	18.62	
"	Stilbestrol+thiouracil (.50 gm.)	10	19.00	
"	Benzestrol (5 mg.–2.5 mg.)	10	21.47**††	23.9
Corn oil		10	19.96	

** =Significantly ($P<0.01$) different from extracts of tissues from untreated lambs.
†† =Significantly ($P<0.01$) different from untreated rats.

In trial II, only those lambs which were bean-fed and were treated orally with benzestrol (5mg./day for the first 54 days and 1 mg./day for the remainder of the feeding period) retained significant ($P<0.01$) amounts of estrogenic materials in the portions of their carcasses assayed.

In trial III, once again only the carcasses of the benzestrol-fed lambs contained detectable amounts of estrogenic residues ($P<0.01$).

The presence of residual estrogenic activity in the carcasses of the stilbestrol-fed lambs in trial I, and its absence from the stilbestrol-fed groups in trial II is probably due to the lower levels at which the hormone was fed during the latter part of trial II. In trial I each lamb received 2.5 mg. per day for the last 33 days of the experiment, whereas in trial II no lambs received more than 1 mg. per day during the last 7 weeks of the trial. The levels of stilbestrol fed in trials I and III were comparable, and the failure to find significant residual estrogenic activity in the carcasses of the stilbestrol-fed lambs in trial III may possibly be attributed to the greater variability of the assay animals used in this trial.

The discovery of residual estrogens in the carcasses of benzestrol-fed

lambs in trials II and III when stilbestrol-fed lambs did not contain detectable amounts was unexpected since stilbestrol is several times more potent than benzestrol insofar as rat uterine stimulation is concerned (Armstrong, 1957). This suggests different pathways of metabolism for the two preparations, or at least that benzestrol is inactivated much more slowly than stilbestrol, and therefore accumulates in the tissues more rapidly.

TABLE 5. MEAN WEIGHTS OF VARIOUS ORGANS AND GLANDS FROM HORMONE-TREATED LAMBS

Treatment	Ewes		Wethers	
	Uterus	Both ovaries	Seminal vesicles	Bulbo-urethrals
	gm.	gm.	gm.	gm.
		Trial I[a]		
Control	11.0	0.92	0.49	0.27
Progesterone-estradiol	11.7	1.09	1.36	1.82
Stilbestrol	14.1	0.65	3.41	2.91
		Trial II[b]		
Control	....	1.14 (4)	0.70 (2)	0.54 (1)
Stilbestrol (5–1)	....	0.63 (1)	3.15 (6)	4.98 (7)
Stilbestrol (5–0)	....	1.08 (5)	1.78 (3)	2.62 (3)
Benzestrol	....	0.64 (3)	1.83 (3)	1.72 (3)
		Trial III[b]		
Control	13.8 (5)	1.3 (5)	1.2 (4)	1.0 (1)
Stilbestrol	15.3 (3)	0.9 (3)	4.6 (3)	4.3 (3)
Stilbestrol+thyroprotein	10.9 (3)	0.7 (3)	4.9 (3)	3.5 (3)
Stilbestrol+thiouracil	14.0 (4)	1.2 (4)	3.4 (3)	2.4 (3)
Benzestrol	12.6 (3)	0.4 (3)	3.9 (3)	2.8 (3)

[a] Data represent the average of four weights.
[b] Figures in brackets indicate the number of weights per average.

More research is necessary before it can be certain how much significance, if any, should be attached to estrogenic residues of the order of magnitude reported in these experiments. Calculations for an average carcass of the stilbestrol-fed group of lambs in trial I (the group found to retain the greatest amount of estrogenic material) indicate a total estrogenic residue per 43 lb. carcass equivalent to only 0.29 mg. of stilbestrol. Thus a human would need to consume almost two lamb carcasses per day in order to obtain a daily stilbestrol intake of 0.5 mg., an amount which is considered by Goodman and Gilman (1955) to be a low oral therapeutic daily dose. At the same time, the effects of continued ingestion of even these small amounts of the hormone over a prolonged period of time are not known.

More information is also necessary concerning the amount of estrogen which may be found in carcasses of normal animals slaughtered while in estrus or during pregnancy. Koch (1956) has stated that the flesh of pregnant animals often contains larger quantities of estrogen than that of animals which have been treated with synthetic estrogens. Leguminous plants such as alfalfa contain significant amounts of estrogens under certain conditions. Alfalfa meal assayed in this laboratory has been found to contain estrogenic activity equivalent to approximately 3.5 micrograms stilbestrol per kg. In addition, Stob, Davis and Andrews (1957)

TABLE 6. WEIGHTS OF ADRENAL, THYROID AND PITUITARY GLANDS FROM LAMBS IN TRIAL III

Treatment	Adrenals	Thyroid	Pituitary
	gm.	gm.	gm.
		Ewes[a]	
Control	2.5	4.3	0.52
Stilbestrol	2.7	3.2	0.57
Stilbestrol+thyroprotein	3.4	4.3	0.57
Stilbestrol+thiouracil	3.2	3.4	0.57
Benzestrol	2.8	3.2	0.53
Average	2.9	3.7	0.55
		Wethers[b]	
Control	3.5	3.1	0.52
Stilbestrol	4.3	2.2	0.87
Stilbestrol+thyroprotein	3.4	2.5	0.67
Stilbestrol+thiouracil	3.2	3.1	0.73
Benzestrol	3.6	3.4	0.83
Average	3.6	2.8*	0.72*

* Sex difference significant at the 5 percent level.
[a] Average weight of 18 glands.
[b] Average weight of 16 glands.

and Pieterse and Andrews (1956) have detected considerable amounts of estrogenic substances in many varieties of alfalfa at various stages of maturity. It is not known whether these plant estrogens contribute to the estrogenic activity of the meat of animals consuming them.

Physiological Side Effects. Tables 5 and 6 present data showing the variation in gland weights caused by hormone supplements. Stilbestrol and benzestrol feeding decreased the weights of the ovaries. The addition of thyroprotein to stilbestrol did not alter this effect, but in thiouracil-treated lambs the ovarian weights were similar to those from untreated ones. The uteri from stilbestrol-fed ewes were heavier, while those from stilbestrol-thyroprotein and benzestrol-fed lambs were lighter than those

from controls. Other treatments caused little change in uterine weights. All estrogen treatments caused marked increases in weights of the bulbourethrals and seminal vesicles as have been reported by Clegg *et al.* (1955). The progesterone-estradiol combination increased the accessory gland and uterine weights to a lesser degree than did stilbestrol. Thiouracil also depressed the stimulating effect of stilbestrol on uterine and accessory gland weights. Although 3 wethers in the progesterone-estradiol group (trial I) and 1 wether each in the stilbestrol and stilbestrol-thyroprotein groups (trial III) were removed because of urinary blockages, the increased size of accessory glands was not believed to have been a causative factor since post mortem examinations showed large amounts of a crystalline material in the kidneys, urethrae and bladders of these lambs.

In trial III adrenal, pituitary and thyroid glands were examined.

TABLE 7. AVERAGE THYROID FOLLICLE CELL HEIGHT (MICRA)

	Corn	Cooked bean	Average
Control	7.17	6.90	7.04
Stilbestrol	9.69	8.88	9.29
Stilbestrol+thyroprotein	7.28	5.62	6.45
Stilbestrol+thiouracil	8.62	9.07	8.85
Benzestrol	8.23	6.53	7.38
Thyroprotein	5.74	....	....
Average	8.20	7.40	....

Clegg *et al.* (1955) reported increased size of pituitary and adrenal glands in stilbestrol-implanted lambs. This was also the case in the present study, as adrenal weights were slightly increased and pituitary weights significantly increased in the stilbestrol-fed lambs. The increase in pituitary weight as a result of feeding either stilbestrol or benzestrol occurred in wethers but not in ewes (table 6). Microscopic examination of sections of adrenals revealed no gross changes in the histology of these glands. Hormone treatment generally caused a decrease in thyroid weights, and stilbestrol was the most effective in this respect. Differences in thyroid weights between treatments were not statistically significant, but the thyroids of ewes were significantly heavier than the thyroids of wethers.

Examination of thyroid sections showed that follicular epithelial cell heights, an index of secretory activity, were greatest in thyroids from stilbestrol-fed lambs and least in those from lambs fed stilbestrol plus thyroprotein. Addition of thiouracil to stilbestrol in the lamb ration also increased follicle cell height, but the follicles were larger and contained more colloid than those from stilbestrol-fed lambs.

Table 7 lists the mean thyroid follicle cell heights for lambs on each

treatment. The mean thyroid epithelial cell height for two lambs fed thyroprotein alone is also included for comparative purposes. These averages were obtained from measurements of two cells in each of five follicles in each of three sections. Analysis of variance indicated highly significant ($P<0.01$) differences in cell heights due to treatment. In addition, the follicular cell heights of thyroids from corn-fed lambs were significantly ($P<0.05$) greater than from bean-fed lambs. This increase in thyroid activity of estrogen-treated lambs may be an effect, rather than a cause, of the increased feed consumption and growth rate of these lambs. Thyroid epithelial cell heights have been found to increase in heifers with increasing levels of feeding (Sorensen *et al.*, 1958).

Other physiological side effects of estrogen supplementation found in these trials were similar to those reported by many other researchers in this field. Varying degrees of teat and udder development were found in all treated groups, the stilbestrol-fed lambs showing the greatest stimulation, but these changes were not of great magnitude in any case.

Summary

Estrogen bioassays were conducted on extracts of tissues from lambs implanted with progesterone and estradiol, and lambs fed stilbestrol or benzestrol, or combinations of either thiouracil or thyroprotein with stilbestrol. Measurable amounts of estrogenic substances were found in the livers of lambs fed stilbestrol or lambs implanted with progesterone plus estradiol, and in lean meat from these implanted lambs.

In one of two trials, a small but statistically significant amount of estrogenic activity was found in the fat from lambs fed stilbestrol at the rate of 5 and 2.5 mg. per day for the first and second halves of the feeding period, respectively.

In another trial, in which the level of stilbestrol was reduced from 5 mg. per day to 1 mg. per day at approximately the middle of the feeding period, no measurable estrogenic activity was found in the meat.

In two trials detectable amounts of estrogenic activity were found in the meat of lambs fed benzestrol.

All hormone treatments resulted in increased size of accessory sex glands of wethers and in most cases in increased uterine weights and decreased ovarian weights of ewes. Pituitary glands of wethers on all hormone treatments were considerably heavier than those of untreated wethers, but hormone treatments did not affect pituitary weights of ewe lambs.

Highly significant differences due to hormone treatments were found in thyroid activities as assessed by thyroid epithelial cell heights. The thyroid epithelial cell heights in decreasing order according to hormone treatment were as follows: stilbestrol, stilbestrol plus thiouracil, benzestrol, untreated, stilbestrol plus thyroprotein and thyroprotein.

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A STUDY OF STILLBIRTHS IN A HERD OF RANGE CATTLE[1]

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THERE is little published information pertaining to stillbirths in beef cattle. However, data accumulated over a period of years at the U. S. Range Livestock Experiment Station, Miles City, Montana, indicate that stillbirths are responsible for considerable economic loss under the typical range environment in which a large proportion of our cattle population is produced.

Although this paper does not include some of the more critical data from autopsies and blood analyses currently being obtained, it is hoped that these herd histories, including a relatively large number of births, may stimulate interest in this problem. Since the sample was composed of both inbred and noninbred herds it was possible to obtain some measure of the effects of inbreeding on the frequency of stillbirths.

Source of Data. Stillbirths occurring during the period 1936-57 were studied in the inbred and noninbred Hereford herds maintained at the station. Stillbirths were arbitrarily classified as those in which there was no evidence of movement by the calf after birth, although in a number of cases an autopsy disclosed functional lungs. Only those stillbirths occurring within the range of live births were considered. In no case was brucellosis, leptospirosis or vibriosis thought to be the causative agent. In recent years blood tests were made on dams giving birth to stillborn calves. All tests were negative for brucellosis, leptospirosis and vibriosis. Calfhood vaccination for brucellosis has been practiced in this herd ever since the vaccine was made available and brucellosis tests have been made at approximately three-year intervals.

Results and Discussion

Stillbirths from 1936-57, inclusive, totalled 316 from 8857 births, or 3.6%. This figure was higher than the loss from birth to weaning for the same period. McCormick *et al.* (1956) reported a 5% stillbirth loss over an 18-year period in the herd at the Georgia Coastal Plain Experiment Station.

Sex and Weight of Calf. Males comprised 62% of the total stillbirths. The over-all percentage of males was 51. By a chi-square test, the preponderance of male stillbirths was highly significant ($P<0.01$). During the same period more females than males were lost from birth to weaning, but the difference was not statistically significant. McCormick (1957)

[1] A contribution of Western Regional Project W-1 on beef cattle breeding research.

TABLE 1. BIRTH WEIGHTS OF LIVE AND STILLBORN CALVES (1951–57)

Items	Live calves	Stillborn calves
Number of head	3342	158
Mean weight, lb.	78.7	68.7
Standard deviation, lb.	9.24	20.24
Range, lb.	27–112	17–127

listed 33 males and 10 females among stillbirths in the Polled Hereford herd at the Georgia Coastal Plain Experiment Station for the period 1937-57, inclusive, or 77% males.

Dystocia is often attributed to heavy birth weights. Although all losses from dystocia may not have been identified, it appears that heavy birth weight was not a major cause of stillbirth in the study herds. As is shown in table 1, the stillborn calves averaged 10.0 lb. lighter at birth, had a larger standard deviation and a wider range in birth weights than did the calves born alive.

The number of stillborn calves above and below the mean birth weight of live calves born during the same period are listed in table 2. Segregations were made by year and sex, and are presented by sequence of calving.

Sixty-seven percent of the stillborn calves shown in table 2 were below the mean birth weight of live calves. A significantly high proportion ($P<0.01$) of the stillbirths occurring in the "All others" class was below the mean birth weight of live calves. Data presented by McCormick (1957) indicate that heavy birth weight was not the major cause contributing to stillbirths in the Georgia Coastal Plain Experiment Station herd. Of 43 calves reported, 23 were below the mean birth weight of all calves, the data being adjusted for sex and age of dam.

Sequence and Time of Calving. Since birth of a stillborn calf, per se, was not used as a reason for culling it was possible to compare the incidence of stillbirths between first calves and subsequent calves from the same dams. Sequence of birth proved to have a highly significant ($P<0.01$) effect upon the incidence of stillbirths (table 3).

TABLE 2. NUMBER OF STILLBIRTHS ABOVE AND BELOW MEAN BIRTH WEIGHT[1] OF LIVE CALVES LISTED BY SEQUENCE OF CALF (1951–57)

Calving sequence	Above mean birth weight		Below mean birth weight	
	Number	Percent	Number	Percent
First calf	32	46	37	54
All others	20	22	69	78

[1] Sex and age adjusted within years.

TABLE 3. STILLBIRTH INCIDENCE AMONG FIRST CALVES COMPARED TO SUBSEQUENT CALVES (1936–57)

Calving sequence	All births	Stillbirths	Percentage Incidence
First calf	2420	163	6.7
All others	6437	153	2.4

The 316 stillbirths were classified by year and in reference to the median calving date. It was found that they were quite uniformly distributed throughout the calving season. A total of 157 stillbirths occurred before, 156 after, and 3 on the median calving date.

Inbreeding Effect. One line of cattle was maintained as a closed herd throughout the period covered by the study. Other lines have been added at varying intervals. A relatively mild system of inbreeding has been practiced in the development of all lines. F_x values generally range 15-20% at the present time. A comparison of these groups with a test population, graded to all lines and maintained under similar environmental conditions, offers some insight into the effect of inbreeding upon stillbirth incidence. Inbreeding coefficients have not been calculated in the test herd but are known to be considerably lower than in the line herds. In table 4 are shown the number of stillbirths in Line 1, in a composite of all other inbred lines, and in the test herd.

A significantly higher incidence ($P<0.01$) occurred in both inbred populations than in the test herd. Although differences between lines and between years were statistically significant, the total variance attributable to these two effects was very low compared to the variance within subclasses.

Craft (1953) reports 1.6 percentage points increase in stillborn pigs resulted with an increase of 10 percentage points in the inbreeding of the dam. Because of the comparatively fewer stillbirths and less intensive inbreeding it was not possible to make similar estimates from these data. There was a trend towards an increased incidence of stillbirths associated with higher levels of inbreeding, as is shown in tables 5 and 6. The distributions of live and stillbirths were significantly different ($P<0.01$), when grouped by either Fx of dams or Fx of calves. There were insufficient

TABLE 4. STILLBIRTH INCIDENCE IN INBRED LINES AND A TEST HERD (1936–57)

Herd	All births	Stillbirths	Percentage Incidence
Line 1	1822	82	4.5
All other lines	2708	114	4.2
Test herd	4261	120	2.8

TABLE 5. INCIDENCE OF STILLBIRTHS IN LINE 1 BY INBREEDING OF DAM (1936–57)

Item	Fx of dams					
	0–6	7–12	13–18	19–24	25–30	31–)
Number of live births	611	334	584	186	15	10
Number of stillbirths	12	17	35	12	5	1
Percent stillbirths	1.9	4.8	5.7	6.1	25.0	9.1

numbers in the other lines to make a similar appraisal of inbreeding effect upon stillbirth incidence.

Other Factors. There are a number of factors that may contribute to the incidence of stillbirths in beef cattle. Hart and Guilbert (1933) reported the birth of dead or weak calves resulting from vitamin A deficiency. Several lethals may result in stillbirths. The most commonly recognized is the type of dwarfism reported by Johnson *et al.* (1950). Dystocia and malpresentation of the fetus are other common causes of stillbirths. Listed in table 7 are the identified factors associated with stillbirths recorded in this herd.

Dystocia was used in a relatively narrow sense to cover those births in which difficult labor was due to such factors as size of calf and pelvic abnormalities of the dam. Dystocia has been discussed previously in terms of size of calf. Since it has been a consistent management practice at this station for the cows to be bred to calve at approximately 36 months, dystocia due to size of cow has not been a serious problem. Losses due to dystocia were recorded in 12 of the 13 lines maintained at the station in within line matings.

Williams (1948) quotes references stating that 95% of ruminant births are anterior presentations. Similar information was not obtainable under the conditions of this study. Benesch and Wright (1952) state that the relative incidence of dystocia is higher in posterior presentations than in anterior and that a consideration of the course of parturition would indicate that posterior presentation is abnormal. The percentage of stillbirths in this study known to have resulted from posterior presentations indicate that unassisted live births occur proportionately less frequently in pos-

TABLE 6. INCIDENCE OF STILLBIRTHS IN LINE 1 BY INBREEDING OF CALF (1936–57)

Item	Fx of calves					
	0–6	7–12	13–18	19–24	25–30	31–)
Number of live births	189	338	777	311	80	45
Number of stillbirths	8	12	27	19	10	6
Percent stillbirths	4.1	3.4	3.4	5.8	11.1	11.8

terior presentations. Further, the incidence of posterior presentations and consequent losses from one sire used two successive years in an inbred line were high enough to suggest an hereditary factor conditioning such malpresentations. A total of 10 stillbirths listed among 198 total births in this line were known to be posterior presentations.

The fetal malpostures listed were those in which the calf was born with one or more limbs or the head out of position or in which there was evidence of body torsion.

Of 70 twin calves, 11 were stillborn in the study herd during the period 1936-57, inclusive. Twinning occurred in approximately 0.5% of the total births.

TABLE 7. IDENTIFIED FACTORS ASSOCIATED WITH STILLBIRTHS (1936–57)

Factors	Number of stillbirths	Percentage of total
Dystocia	50	37.0
Posterior presentation	35	25.9
Malposture of fetus	9	6.7
Twinning	11	8.1
Abnormal fetus	30	22.2

All calves exhibiting acute skeletal abnormalities were listed in the last group. There were no birth weights over 50 lb. Undoubtedly some of these cases were lethals. Only one calf was classified as a typical dwarf as described by Johnson *et al.* (1950). Of course, the possibility exists that other stillbirths were due to the same lethal since Gregory (1955) reported a rather wide range of phenotypic variation. There is also a possibility that other thus far unrecognized lethals are responsible for some of the stillbirths, particularly in this group.

Summary

Stillbirths occurring in the beef herds at the U. S. Range Livestock Experiment Station totalled 316 from 8857 births (3.6%), for the period 1936-57 inclusive. Although there did not appear to be any single major causative factor the following characteristics were noted in the stillborn calves:

A significantly higher number of males than females were stillborn. Live calves averaged heavier at birth, deviated less from the mean and fell within a narrower birth weight range than did stillborn calves. A significantly higher number of stillborn calves were recorded during first births than in subsequent births, and a significantly higher proportion were above the mean birth weight. A significantly higher number of calves were stillborn in the inbred population than in the test population of the study

herds. Posterior presentations and twinning resulted in a proportionately higher than average percentage of stillbirths.

Few lethals were identified but the lack of a positive diagnosis of causative factors in a number of the stillbirths leaves this problem open to question.

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RELATION OF HEAT TOLERANCE DETERMINATIONS TO PRODUCTIVITY IN BEEF CATTLE

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THE purpose of this study is to report the correlations between heat tolerance and production in beef cattle at the Iberia Livestock Experiment Farm near Jeanerette, Louisiana, from 1942 to 1951 inclusive. The present study supplements the work by Rhoad (1944) and by Baker and Black (1950) by ascertaining the relationship between heat tolerance, as measured by respiration rates and body temperatures, and production. Production was measured by weights of dams and their progeny at birth, at 6 months and at 5 years of age.

Materials and Methods

Initial heat tolerance experiments led to the development of a formula (Rhoad, 1944) for use in calculating heat tolerance coefficients on an individual basis. This formula, known as the Iberia heat tolerance test for cattle, is as follows:

Coefficient of heat tolerance $= 100 - 10\ (BT - 101)$ in which BT = average body temperature based on rectal temperature readings (made at 10 a.m. and 3 p.m. on three different days) with the average air temperature 85° F. or more (cattle held in unprotected area during test period, with access to water, but having no shade); 101 = normal body temperature of cattle; 10=a factor to convert degrees' deviation in body temperature from the normal to a unit basis; 100 = perfect efficiency in maintaining temperature at 101° F.

In the present study, heat tolerance data from 1942 through 1951 were analyzed on a within year, breed and age basis. Simple correlation and regression coefficients (Snedecor, 1946) were computed between the individual cow's heat tolerance coefficient and her birth weight, 6-months weight and 5-years weight, if available; also with the average birth, six-months and 5-years weights of her calves when available. Weights were adjusted for age of dam and for sex of calf. F tests (Snedecor, 1946) showed a significant difference between results when unadjusted rectal temperatures were used and when those which had been corrected to an air temperature of 90.0° F. were used; consequently, all of the body temperatures were corrected on a within year basis and the heat tolerance coefficients used in the analyses came from adjusted temperatures.

Respiration rates also were adjusted to correspond to an air temperature

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of 90.0° F. using simple regression methods on a within year basis. Correlation coefficients were computed using both adjusted and unadjusted data. Results were essentially the same. The heat tolerance coefficients of wet cows derived from data taken when the cows were 5 years old were used if available. If no heat tolerance coefficient on a "wet" cow basis was available at 5-years and one was available at 6 years, the latter was used and so on. If no heat tolerance coefficients were available at 5 years or older and a wet-cow coefficient was available at 4 years, it was used. If data were unavailable from the above years, coefficients derived from combining one- and two-year data were used, if available. Data from three-year-old dams were used only if no other data were available. Cows were grouped by year and breeding.

In 1947 Baker concluded that the average of the heat tolerance coefficients taken at one and two years constituted a statistically significant prediction of mature wet-cow heat tolerance. Beginning in 1948 heat tolerance ratings were made on yearling and two-year-old heifers and on two-year-old bulls only.

Results and Discussion

The regressions of dam's heat tolerance coefficient on, and correlations with, production as measured in this study were not significant. The correlations between the heat tolerance coefficients of 258 Brahman x Angus cows with 239 degrees of freedom and the cow's own birth weight was –0.05. The regression value was –0.32 lb. When cows' 6-months weights were used, the correlation value was –0.06; the regression value, –0.40. Correlations between the heat tolerance coefficients of 230 Brahman x Angus cows (211 degrees of freedom) and the average birth weights of their calves was –0.04. The regression value of these birth weights on the heat tolerance coefficient was –0.04 lb. When the average 6-months weights of 225 calves (206 degrees of freedom) were considered, the correlation was 0.09 and was positive, the regression was 0.42. Data from 56 Africander x Angus crossbred cows (46 degrees of freedom) were equally inconclusive. Consequently the regression coefficients have little or no predictive value, even though they are large enough to be of economic importance. While it is possible that the use of multiple correlation methods might have given a little more information, it is doubtful if it would have been sufficient to change any finding.

If respiration rates could be used as a measure of heat tolerance instead of body temperatures, considerable time and effort would be saved, and wear and tear on men and cattle avoided. For this reason correlations between respiration rates and rectal temperatures were computed. They were positive and highly significant in all groups except mature Brahman cows which were negative and not significant. Correlation coefficients for all

other cattle studied ranged from approximately 0.5 to 0.6. These correlations are not considered sufficiently high to justify the use of respiration rates as a measure of body temperature or of heat tolerance. Correlations of respiration rates with rectal temperatures of the various animals tested are given in table 1.

The correlation of respiration rates with performance was calculated using data from Brahman-Angus females only. Results were similar to those obtained using heat tolerance coefficients.

The smallness of the correlations between rectal temperatures or respiration rates and the performance traits studied indicate that ability to

TABLE 1. CORRELATIONS OF RESPIRATION RATES WITH RECTAL TEMPERATURES

Breeding	Yearlings			Two-year-olds			Cows 3 yrs. and older		
	n	df	r	n	df	r	n	df	r
Brahman	102	78	0.58**	77	65	0.43**	60	36	—0.14
Afr. × Ang.	485	343	0.56**	294	188	0.62**	616	556	0.51**
Bra. × Ang.	1926	1772	0.46**	1224	1042	0.51**	1882	1670	0.54**
Aberdeen-Ang.	....	....	....	....	....	....	120	84	0.80**
Santa Ger.	....	....	....	....	....	....	30	18	0.62**

** Significant at the 1% level.

maintain normal temperatures and low respiration rates probably is only one of the traits needed for good performance under Jeanerette conditions. The correlations are not significant statistically, but even if additional data should establish their significance, they are too low to be used to predict performance. Direct selection for performance should result in selection pressure not only for heat tolerance but for other characteristics necessary for high performance.

Even if smaller animals are more heat tolerant than larger animals, as might be faintly suggested by the number of negative correlations, these correlations, if real, are so small as to present little obstacle to selection for heavier weights.

Summary and Conclusions

Analyses of heat tolerance data at the Iberia Livestock Experiment Farm, Jeanerette, Louisiana, for the years 1942 to 1951 inclusive showed highly significant correlations between respiration rates and body temperatures in beef cattle. Correlations for crossbred cattle ranged from approximately 0.5 to 0.6. These correlations are not considered sufficiently high to justify the interchangeable use of respiration rates and body temperatures as a measure of heat tolerance.

Correlations between dam's coefficient of heat tolerance and production as measured by her own weight at birth, 6 months and 5 years, and

those of her progeny at the same ages were not statistically significant and were numerically too low to have much predictive value. In general the same was true when respiration rates were used instead of heat tolerance coefficients.

As a consequence of these findings, use of heat tolerance ratings as aids to selections in cattle at the Iberia Livestock Experiment Farm has been discontinued. Apparently selection on the basis of production automatically includes sufficient selection for necessary heat tolerance.

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THE EFFECT OF CARCASS GRADE, WEIGHT AND CLASSIFICATION UPON BONELESS BEEF YIELD [1]

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PRODUCTION of boneless, kitchen-ready meat cuts, especially those handled in a frozen state, seems destined to increase markedly. This type of preparation, performed in the packing plant, would decrease bulk and weight of meats, thereby reducing transportation costs, and would provide for more efficient use of trimmings and of cutting by-products. Decreased weight and bulk of food products becomes especially important in some military situations.

This type of meat preparation has become important very recently; therefore, few data exist on cutting yields. This study was conducted to determine the expected yield of boneless, kitchen-ready beef and the possible effect of grade, carcass weight and class upon this yield. It is felt that this study would also provide data that would be extremely helpful as reference material.

Materials and Methods

Beef carcasses for this study were selected to fit the middle of either the Choice, Good or Commercial (and Standard) grade, by a representative of the U.S.D.A. Meat Grading Service. Carcasses and cutting data were obtained from carcass groups representing the aforementioned grades in the steer, heifer and cow classifications and 400–500, 600–700 and 800–900 lb. carcass weight groups, where possible. If obtainable, 20 carcasses were processed in each experimental group. (See table 1)

The carcasses were boned and processed no less than 48 hours and no more than 96 hours from time of slaughter. At time of boning, the internal temperature of the round did not exceed 42° F. Carcasses were ribbed between the twelfth and thirteenth ribs. The forequarter was cut canner style. The foreshank with arm bone (humerus) and arm bone muscles attached was removed at the shoulder knuckle joint (humerus—scapula

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socket) and the shoulder clod was removed canner style by stripping from the lateral surface of the scapula. The thin end (dorsal portion) was removed by making a cut at right angles to the anterior side and separating the trapezius muscle from the infraspinatus muscle at the point where the two muscles overlap. The latissimus dorsi lying over the rib section was removed with the clod by a cut parallel to the anterior side, starting at the point where the latissimus dorsi meets the thicker armbone (triceps) muscle. The rib was separated from the chuck between the third and fourth rib, while the short plate and brisket were removed by making a cut extending from approximately 2 inches ventral to the scapula and humerus socket to a point twice the width of the longissimus dorsi from the distal edge of the longissimus dorsi at the loin end of the rib. The brisket was separated from the plate between the fifth and sixth rib. The short plate was trimmed for use as ground beef, whereas the brisket was divided into boneless brisket, deckle and trimmings. The rib section was divided into the rib eye, rib cover (trapezius), wing and cap (all remaining intact muscles), and trimmings. The chuck was divided into chuck fillet (supraspinatus), chuck rolls, inside chuck and trimmings. The chuck fillet was removed in one piece in the regular manner. The neck was separated from the chuck by a cut parallel to the rib side and coming between the fifth and sixth cervical vertebrae. After removal of the remaining ribs and vertebrae by scalping, the chuck roll and inside chuck were separated by cutting parallel to the back line at a point where the heavy muscles of the chuck fade out and join the thinner inside chuck. The chuck roll was separated into a neck and blade portion at a point that was originally just anterior to the first rib.

The kidney knob, hanging tender and flank were removed in the conventional manner. The tenderloin was removed in the conventional manner and surface fat in excess of ½ inch was removed. The round was removed canner style, as close as possible to the pelvis, in such a manner as to leave the maximum amount of lean meat on the inside of the round and to produce a full cut knuckle. The short loin was separated from the sirloin by cutting at right angles to the back line at a point just anterior to the ilium (hip bone). The rump was removed from the sirloin by cutting on a line between the hip joint and the posterior surface of the fifth sacral vertebra. The round was divided into top round, bottom round with heel attached, knuckle and trimmings. The facing, knuckle bone (patella) and tendon end were removed from the knuckle, while the tensor fascia latae was considered a part of the bottom sirloin butt. The heel was cut from the bottom round by making a cut parallel to the rump end at the base of the fat pocket surrounding the popliteal lymph gland immediately adjacent to the gastrocnemius muscle. The loin was divided into top sirloin butt, bottom sirloin butt, loin strip, loin wing (obliquus abdominii and transversus abdominus) and trimmings. The top and bottom sirloin butt

were separated by cutting along the seam immediately adjacent to the heavy fat deposit.

The following chart indicates the utilization of each cut:

Roast, dry heat	Steaks, moist heat	Ground
Top round	Bottom round	Hanging tender
Knuckle	Chuck roll (blade end)	Flank
Rump	Chuck fillet	Armbone muscles
	Inside chuck	Shank meat
Roast, moist heat	Flank steak	Loin wing
	Rib eye cover	Rib wing
Heel	Bottom sirloin butt	Short plate
Chuck roll (neck end)		Skirt
Clod	**Steaks, dry heat**	Neck
Brisket		Deckle
	Top sirloin butt	Rib fingers
	Loin strip	Chuck facings
	Tenderloin	Trimmings
	Rib eye	

All cuts were completely boned and all bone slivers, cartilage pieces, blood clots and bruised portions were removed. All cuts assigned for roasts or steaks had the surface fat trimmed so as not to exceed ½-inch in thickness. Semi-attached fat and tag ends of lean were removed from cuts assigned for roasts or steaks. Dry heat steaks were cut into 6-oz. portions with a ¾ oz. tolerance. All connective tissue on the surface of boneless cuts for steaks was removed. Moist heat steaks were cut from ½ to 1 inch in thickness with a minimum weight allowance of 5½ oz. Moist heat steaks were run through a mechanical tenderizer one time, but smaller pieces were not knitted together. Cutting, boning, and steak slicing operations were continuous. Each boneless cut and the kitchen-ready steaks from each carcass were weighed, as soon as possible, to the nearest ounce. The percentage yield of each cut was calculated, based on total boneless beef yield (two steak and two roast categories plus ground beef). The boneless beef yield, fat, and bone were calculated as percentages of total carcass weight. Final fat percentage included fat trim plus or minus fat from ground beef when adjusted to 25% analytical fat. Yield of ground beef was adjusted plus or minus so that the final product would be calculated to contain exactly 25% analytical fat. Data were accumulated separately for each carcass used in the study.

Analysis of variance was used to determine if differences existed between grades, classes and weight groups with regard to boneless beef to bone ratio, and percentages of boneless beef, fat, bone, dry heat steaks, dry heat roasts, moist heat steaks and moist heat roasts.

Results and Discussion

Table 1 contains the percentages of fat, bone and boneless beef based on carcass weight. The fat percentage includes all fat trimmings plus or

TABLE 1. BEEF YIELD DATA [a]

Grade	Class	Carcass weight cwt.	No. of carcasses	% Fat [b]	% Bone	Boneless beef yield	BBY/ bone [c]	% Top sirloin butt	% Loin strip	% Tender-loin	% Rib eye	% Total grill steaks [d]	% Top round	% Rump	% Knuckle	% Total oven roasts
Ch.	Steer	4–5	20	17.17	15.70	64.99	4.16	4.21	4.11	2.09	4.30	14.71	7.31	2.09	4.92	14.32
Ch.	Steer	6–7	20	19.61	14.23	64.19	4.51	4.09	4.02	2.02	4.27	14.40	7.22	2.16	4.51	13.89
Ch.	Steer	8–9	20	24.11	13.66	60.03	4.39	4.17	3.92	1.95	4.45	14.49	7.09	2.18	4.77	14.04
Ch.	Heifer	4–5	20	20.11	14.71	63.43	4.32	4.47	4.26	2.16	4.11	15.00	7.32	2.26	4.98	14.55
Ch.	Heifer	6–7	20	27.16	13.38	57.63	4.32	4.23	4.11	2.06	4.71	15.11	7.21	2.09	4.97	14.28
Ch.	Cow	4–5	2	21.17	14.12	63.34	4.49	4.62	3.97	1.88	4.49	14.96	7.25	2.03	5.14	14.42
Ch.	Cow	6–7	20	24.91	13.30	59.73	4.50	4.48	4.08	2.01	4.65	15.22	7.07	2.11	4.86	14.04
Gd.	Steer	4–5	20	15.18	16.90	65.78	3.91	4.36	3.95	2.05	4.08	14.45	7.29	2.21	5.01	14.51
Gd.	Steer	6–7	20	16.25	15.89	65.73	4.14	4.24	3.85	2.12	4.27	14.48	7.40	2.23	4.79	14.42
Gd.	Steer	8–9	20	18.08	15.28	64.65	4.24	4.11	3.79	1.90	4.24	14.04	6.97	1.96	4.75	13.68
Gd.	Heifer	4–5	20	16.42	16.27	65.53	4.04	4.20	4.01	2.20	4.02	14.42	7.34	2.36	5.01	14.71
Gd.	Heifer	6–7	20	21.96	14.20	61.80	4.37	4.30	3.94	2.03	4.39	14.67	7.29	1.95	5.04	14.28
Gd.	Cow	4–5	4	19.70	15.36	63.00	4.11	4.51	4.19	2.06	4.53	15.29	7.40	1.97	5.07	14.45
Gd.	Cow	6–7	20	22.14	14.28	61.41	4.32	4.54	4.02	2.02	4.32	14.91	7.11	2.01	5.05	14.16
Com'l	Steer	4–5	21	11.35	18.89	67.72	3.60	4.17	3.76	2.13	4.02	14.09	7.46	1.95	5.40	14.81
Com'l	Steer	6–7	20	13.22	17.19	67.64	3.95	4.18	3.82	2.00	4.06	14.07	7.09	1.91	5.12	14.12
Com'l	Steer	8–9	1	10.78	17.92	68.71	3.83	4.39	3.48	1.75	3.98	13.61	7.14	2.06	4.04	13.24
Com'l	Heifer	4–5	14	12.18	17.74	67.90	3.83	4.38	3.93	2.21	4.23	14.75	7.67	2.06	5.55	15.28
Com'l	Cow	4–5	20	12.84	17.32	67.97	3.92	4.28	3.84	2.17	4.13	14.42	7.53	1.96	5.26	14.79
Com'l	Cow	6–7	20	20.31	15.23	62.42	4.10	4.49	4.02	1.97	4.47	14.94	7.13	1.91	4.88	13.93

[a] All cuts calculated as a percentage of total boneless beef yield except % fat, % bone and % boneless beef yield which were calculated as a percentage of carcass weight.

[b] Fat trim plus or minus fat from ground beef when adjusted to 25% analytical fat.

[c] Ration of boneless beef yield to bone. All boneless beef cuts trimmed so that no surface fat exceeded ½ inch.

[d] All grill steaks were portion-controlled to weigh 6 oz. plus or minus ¾ oz.

TABLE 2. BEEF YIELD DATA [a]

Grade	Class	Carcass weight cwt.	% Bottom round	% Bottom sirloin butt	% Flank steak	% Chuck blade roll	% Chuck fillet	% Inside chuck	% Rib eye cover	% Total swiss steak	% Heel	% Chuck neck roll	% Clod	% Brisket	% Total moist heat roast
Ch.	Steer	4–5	4.68	1.05	0.66	3.01	1.09	1.34	1.49	13.31	2.78	2.63	7.34	2.46	15.21
Ch.	Steer	6–7	4.46	1.30	0.62	3.46	1.05	1.67	1.85	14.41	2.70	3.06	7.27	2.64	15.67
Ch.	Steer	8–9	4.77	1.23	0.65	3.39	1.03	1.54	1.93	14.55	2.72	3.29	7.11	2.78	15.90
Ch.	Heifer	4–5	4.66	1.04	0.69	3.08	1.08	1.51	1.61	13.66	2.90	2.83	7.23	2.61	15.58
Ch.	Heifer	6–7	4.87	1.11	0.68	3.04	1.07	1.42	1.67	13.87	2.86	2.80	7.39	2.56	15.61
Ch.	Cow	4–5	5.05	1.24	0.70	2.79	0.96	1.28	1.60	13.61	2.89	2.39	7.02	2.68	14.98
Ch.	Cow	6–7	4.82	1.16	0.64	3.04	1.02	1.34	1.70	13.73	2.67	2.86	7.32	2.64	15.50
Gd.	Steer	4–5	4.65	1.24	0.66	3.18	1.14	1.54	1.61	14.02	2.82	2.80	7.22	2.40	15.23
Gd.	Steer	6–7	4.61	1.26	0.66	3.51	1.11	1.79	1.91	14.85	2.76	3.08	7.20	2.57	15.62
Gd.	Steer	8–9	4.77	1.16	0.62	3.51	1.09	1.58	1.83	14.56	2.60	3.07	6.97	2.40	15.05
Gd.	Heifer	4–5	4.70	1.23	0.69	2.86	1.07	1.43	1.60	13.50	2.89	2.57	7.35	2.40	15.21
Gd.	Heifer	6–7	5.08	1.09	0.64	3.02	1.09	1.47	1.67	14.07	2.62	2.71	7.32	2.68	15.33
Gd.	Cow	4–5	4.99	1.11	0.59	3.18	1.10	1.31	1.82	14.10	2.76	2.63	7.28	2.48	15.14
Gd.	Cow	6–7	4.78	1.19	0.64	2.97	1.07	1.27	1.59	13.50	2.92	2.65	7.21	2.42	15.20
Com'l	Steer	4–5	4.87	1.12	0.65	3.15	1.19	1.55	1.71	14.25	2.96	2.59	7.20	2.29	15.04
Com'l	Steer	6–7	4.88	1.10	0.62	3.21	1.10	1.49	1.75	14.15	2.69	2.87	7.06	2.39	15.02
Com'l	Steer	8–9	4.59	1.53	0.67	3.71	1.20	1.88	2.03	15.66	2.61	3.82	7.10	2.68	16.21
Com'l	Heifer	4–5	5.11	1.07	0.69	3.10	1.14	1.49	1.61	14.21	2.83	2.49	7.25	2.37	14.93
Com'l	Cow	4–5	4.86	1.08	0.61	2.92	1.15	1.42	1.53	13.56	2.75	2.44	7.35	2.31	14.85
Com'l	Cow	6–7	4.84	1.08	0.63	3.39	1.11	1.53	1.87	14.44	2.46	2.90	7.26	2.42	15.05

[a] All percentages calculated as a percentage of total boneless beef yield.

minus fat from lean trimmings when adjusted to 25% analytical fat. Ratio of boneless beef to bone is presented to give an estimation of edible meat to bone ratio. These tables also show the percentage of boneless beef represented by each cut. (tables 1 and 2). Totals are presented for the dry heat steaks, dry heat roasts, moist heat steaks and moist heat roasts. These tables should be extremely helpful as reference material.

Statistical comparisons by grade, class, and weight of carcass are presented in table 3. In making the comparison between grades, data were

TABLE 3. YIELD SUMMARIES AND STATISTICAL ANALYSIS [a]

Grade comparison [b]	Com'l	Good	Choice	F. value
Boneless beef yield (% of carcass wt.)	66.07	64.45	62.93	32.64**
Fat (% of carcass wt.)	14.85	17.76	20.71	7.67**
Bone (% of carcass wt.)	17.05	15.71	14.37	145.26**
Boneless beef to bone ratio	3.87	4.10	4.37	81.67**
Dry heat steaks (% of boneless beef)	14.43	14.58	14.81	2.98
Dry heat roasts (% of boneless beef)	14.40	14.43	14.17	5.95**
Moist heat steaks (% of boneless beef)	14.27	14.03	13.83	4.83**
Moist heat roasts (% of boneless beef)	15.02	15.33	15.50	8.60**
Total—steaks and roasts	58.12	58.37	58.31	

Class comparison [c]	Steer	Heifer	Cow	F. value
Boneless beef yield (% of carcass wt.)	65.69	61.31	62.51	37.47**
Fat (% of carcass wt.)	16.19	22.14	20.72	5.94**
Bone (% of carcass wt.)	16.07	14.57	14.71	10.48**
Boneless beef to bone ratio	4.09	4.21	4.25	6.13**
Dry meat steaks (% of boneless beef)	14.34	14.86	15.35	11.96**
Dry heat roasts (% of boneless beef)	14.34	14.50	14.30	1.98
Moist heat steaks (% of boneless beef)	14.53	14.02	13.60	15.83**
Moist heat roasts (% of boneless beef)	15.48	15.35	15.20	2.26
Total—steaks and roasts	58.69	58.73	58.45	

Weight comparison [d]	400–500	600–700	800–900	F. value
Boneless beef yield (% of carcass wt.)	65.39	64.96	62.33	16.11**
Fat (% of carcass wt.)	16.17	17.93	21.11	24.74**
Bone (% of carcass wt.)	16.30	15.06	14.47	41.94**
Boneless beef to bone ratio	4.03	4.33	4.32	19.72**
Dry heat steaks (% of boneless beef)	14.58	14.44	14.26	3.03
Dry heat roasts (% of boneless beef)	14.41	14.16	13.86	4.96**
Moist heat steaks (% of boneless beef)	13.67	14.63	14.56	8.08**
Moist heat roasts (% of boneless beef)	15.22	15.64	15.46	3.52*
Total—steaks and roasts	57.88	58.87	58.14	

[a] All values presented are averages for lots used in comparison.
[b] Comparison includes steers (4–5 and 6–7 cwt.), heifers (4–5 cwt.) and cows (6–7 cwt.).
[c] Class comparison includes choice (6–7 cwt.), good (6–7 cwt.), and commercial (4–5 cwt.).
[d] Weight comparison includes good and choice steers.
* Indicates significance at 5% level of probability.
** Indicates significance at 1% level of probability.

used only from steer carcasses weighing 400–500 and 600–700 lb., heifers weighing 400–500 lb. and cows weighing 600–700 lb., since these are the only groups found in all three grades. For a similar reason, only Choice and Good carcasses weighing 600–700 lb. and Commercial (Standard) carcasses of 400–500 lb. were considered in making the comparison between steers, heifers and cows. In making the weight analysis only data from Good and Choice steers were used. A separate analysis of variance was used when analyzing for differences in grade, in class, and in weight groups. In each analysis, the other two treatment variables were ignored when analyzing for differences in one given variable. The weaknesses of such a procedure are recognized, but a factorial analysis was impossible to perform because of many missing groups.

Boneless beef yield was significantly altered by grade. The highest yield was found in the Commercial grade and lowest in Choice carcasses. Fat percentage was also affected by grade, since the higher grades had more finish. Hankins and Foster (1940) reported similar findings. They found 30.99, 24.48 and 20.37% physically separable fat, respectively, for the Choice, Good and Commercial grades, whereas percentage of separable lean was 52.97, 57.45 and 59.42 with the Commercial carcasses showing the highest value. Kemp *et al.* (1954) also found more physically separable fat in the rib section of Choice carcasses when compared to Good carcasses. Hayton and Aldrich (1956) reported trimmable fat percentages of 11.88, 10.32 and 9.13 for the Choice, Good and Commercial grades. Their percentages were lower than those found in this paper. This may have been partly due to lighter weight carcasses used in their study and to a less stringent fat trimming requirement.

Grade had a very apparent effect on bone weight calculated as a percentage of carcass weight. Our values of 17.05, 15.71 and 14.37%, respectively, for Commercial, Good and Choice carcasses are somewhat lower than the 18.65, 16.23 and 15.38% reported by Hayton and Aldrich (1956) and 20.21, 18.07 and 16.04% found by Hankins and Foster (1940). The results of Hayton and Aldrich may be higher since lighter cattle were used in their study. Hankins and Foster included ligaments and tendons in their percentage of bone figures, therefore they achieved higher values. In all cases, less bone was found in higher grades of beef. Boneless beef yield to bone ratio was also significantly affected by grades. Values of 4.37, 4.10 and 3.87 were calculated, respectively, for the Choice, Good and Commercial grades. Apparently, this is primarily a resultant of the decreasing percentage of bone in the higher grades.

Dry heat steak yield was unaffected by grade, but both dry heat roasts and moist heat steaks exhibited a greater yield in the Commercial and Good grades as compared to Choice. However, moist heat roasts contributed a greater percentage in the higher grades. It is difficult to determine why categories of cuts are affected in this manner. Hayton and Aldrich (1956) used slightly different allocations of cuts to various cate-

gories. They found the yield of dry heat cuts (steaks and roasts) to be lowest in the Commercial grade (21.93%) and highest in the Choice grade (22.60%). Grade affected the total of stew meat and ground component only slightly in their study.

In considering the results of this study, it must be kept in mind that it was completed prior to initiation of the Standard grade. Therefore, those carcasses included in the Commercial grade would now include both Standard and Commercial carcasses. In considering results from tables 1 and 2, one can be reasonably certain that lighter weight Commercial steers and heifers would represent the Standard grade.

Yield of dry heat roasts and moist heat roasts was not affected by classification (sex) of the carcass. However, all other factors were affected ($P = 0.01$). Steers yielded a higher percentage of boneless beef; showed a larger percentage of bone, which resulted in a lower boneless beef to bone ratio, but cut out considerably less fat than either cows or heifers. However, this greater boneless beef yield is reflected only by a similar increase in percentage of moist heat steaks. In fact, steers yielded 14.34% dry heat steaks compared to 14.86% for heifers and 15.35% for cows. Upon looking at table 1, it appeared that this high yield of dry heat steaks in cows was due largely to greater yield of top sirloin butt and rib eye steaks. Kemp *et al.* (1954) and Brown and Branaman (1934) both verified the finding that steer carcasses tend to contain less fat and more lean meat. Brown and Branaman (1934) found the steers' advantage to be a resultant of a greater yield of chuck, round and shank. However, heifer carcasses yielded more rib, loin and rump and showed a smaller percentage of bone. These findings were corroborated by Kennedy *et al.* (1955). Apparently steer carcasses yielded considerably more ground beef component.

Carcass weight exerted a significant effect on yield of moist heat roasts and a highly significant effect on all other factors analyzed except dry heat steaks. Boneless beef yield was markedly decreased in 800–900 lb. carcasses whereas fat content increased as weight increased. Boneless beef to bone ratio was lower in 400–500 lb. carcasses, but similar ratios were found in the other two weight groups. In line with this, Callow (1944, 1950) reported that young beef animals deposited more protein and less fat; therefore, proportion of muscle tissue was found to decrease as live weight increased.

Summary

Boneless beef yield and percentage of bone decreased and fat percentage increased as grade increased when Commercial, Good and Choice beef carcasses were compared. Boneless beef to bone ratio also increased in the higher grades, primarily since bone decreased markedly in higher grades. The increased boneless beef yield in lower grades cannot be attributed to a higher total yield of moist and dry heat steaks and roasts.

Steers, cows and heifers, in that order, showed decreasing boneless beef and bone percentages and increasing fat percentages. The greater boneless beef yield in steers was not attributable to total of moist and dry heat steaks and roasts. Boneless beef to bone ratio was lower in steers, but similar in cows and heifers.

Boneless beef yield was decreased in 800–900 lb. carcasses. Percentage fat was increased and percentage bone decreased as carcass weight increased. Light weight carcasses (400–500 lb.) showed a lower boneless beef to bone ratio; whereas the other two weight groups were similar.

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THE INFLUENCE OF THREE-WEEK WEIGHT ON EIGHT-WEEK WEIGHT OF PIGS WEANED AT THREE WEEKS OF AGE [1]

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DURING the past 10 years, much attention has been given to problems incident to the weaning of pigs at 2 to 21 days of age instead of the customary 56 days. Nearly all of the published reports were concerned with the nutrition of the suckling age pig. In these reports, age had been the determining factor in weaning pigs early.

Jensen *et al.* (1956) reported that each additional pound of pig weight at weaning (12 to 14 days of age) resulted in two additional pounds at 42 or 56 days. Weaning pigs at 7 to 10 days of age or a minimum weight of 5 lb. has been suggested by Speer *et al.* (1954).

Reports relating weaning weights of early weaned pigs to their 8-week weights have been few in number. The objective of this study was to determine the influence of 3-week weight on 8-week weight of pigs weaned at 3 weeks of age.

Materials and Methods

The growth data of 919 spring-farrowed pigs used in early weaning experiments in 1954, 1955 and 1956 at the Minnesota Agricultural Experiment Station provided the material for this study. The pigs were weaned at an average age of 3 weeks and were fed diets composed principally of corn, soybean oil meal, tankage, and dried skim milk and supplemented with adequate vitamins and minerals (Hanson *et al.* 1954, 1955, 1956). Differences in diets consisted of flavors, unidentified growth factor supplements and substitutions of other dried milk by-products for dried skim milk.

The growth data of the pigs were sorted into groups by 3-week weight, sex, breed and year. Weight groupings were: (1) 14.0 lb. and over, (2) 12.0 to 13.9 lb., (3) 10.0 to 11.9 lb., (4) 8.0 to 9.9 lb., (5) 7.9 lb. and under.

The analysis of variance (Snedecor, 1956) was used to test for statistically significant differences among 8-week weights of pigs grouped according to sex, breed and year.

Results and Discussion

A between-within analysis of variance of the 8-week weights of pigs of the breeds represented, of 8-week weights of pigs of opposite sex within

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TABLE 1. AVERAGE WEIGHTS OF PIGS BY SEX AND BREED

Breed	Sex	No. of pigs	3-Week weight, lb.	8-Week weight, lb.
Duroc	Barrow	163	10.49	36.28
	Gilt	188	10.47	37.34
Chester White	Barrow	173	10.41	34.69
	Gilt	137	9.83	33.34
Poland China	Barrow	82	10.18	37.05
	Gilt	78	9.69	34.67
Yorkshire	Barrow	47	11.56	40.95
	Gilt	51	12.28	41.16

each breed, and of 8-week weights of pigs of each breed raised in different years resulted in statistically significant differences between years in the case of the Poland China pigs, and among breeds. No statistically significant differences in the 8-week weights (table 1) of pigs of opposite sex within breeds were found. The 8-week weights of the Poland China pigs raised in 1954 and 1955 (table 2) were significantly ($P<0.01$) different. The relatively poor performance of the Poland China pigs raised in 1955 was due to a reluctance to eat dry feed after they were weaned. Although the 8-week weights of pigs of the breeds represented were significantly different ($P<0.01$), it should not be assumed that the differences in 8-week weights observed in this study are necessarily representative of the breeds

TABLE 2. AVERAGE WEIGHTS OF PIGS RAISED IN DIFFERENT YEARS

Breed	Year	No. of pigs	3-Week weight, lb.	8-Week weight, lb.
Duroc	1954	99	11.10	38.01
	1955	104	10.75	37.11
	1956	148	9.87	35.90
	Av.	351	10.48	36.85
Chester White	1954	174	10.29	34.60
	1955	66	8.94	32.70
	1956	70	10.99	34.15
	Av.	310	10.16	34.09
Poland China	1954	81	10.30	39.61
	1955	79	9.57	32.08
	Av.	160	9.94	35.89
Yorkshire	1955	34	12.48	40.11
	1956	64	11.65	41.57
	Av.	98	11.94	41.06

as a whole. It has been observed frequently that there is considerable variation in growth rate between lines of breeding within a given breed.

The correlation between 3- and 8-week weights of pigs was quite high. From the correlation coefficients presented in table 3, it will be noted that variation in 3-week weights accounts for 48.2 to 76.8% of the variation in 8-week weights of pigs weaned at 3 weeks. These results are much higher than those reported by Donald (1939) where variation in 3-week weights of suckling pigs accounted for 22% of the variation in 8-week weights.

The regression of 8-week weights on 3-week weights indicated that every additional pound, within the weight ranges analyzed, at 3 weeks

TABLE 3. REGRESSION AND CORRELATION COEFFICIENTS OF 3- AND 8-WEEK WEIGHTS OF PIGS

Breed	Regression coefficient	Correlation coefficient
		r
Duroc	2.46	.769
Chester White	2.07	.693
Yorkshire	2.23	.775
Poland China 1954	2.89	.817
Poland China 1955	2.91	.876
All Breeds	2.44	——

resulted in an additional 2.07, 2.23 and 2.46 lb. at 8 weeks for Chester White, Yorkshire and Duroc pigs, respectively.

Because of the highly significant difference between the 8-week weights of the 1954 and 1955 Poland China pigs, regression coefficients were determined for each year. The regression coefficients were virtually the same, 2.89 and 2.91, for 1954 and 1955, respectively.

An average regression coefficient was calculated for the data from all pigs. Because there was no significant difference among the regression coefficients for the various breeds, it is suggested that this regression coefficient is more valid for general use because of the greater number of pigs represented and the greater range of environmental conditions. The average regression coefficient was 2.44.

Summary

The growth data of 919 spring-farrowed pigs were studied to determine the influence of 3-week weight on 8-week weight, when pigs are weaned at 3 weeks of age. The analysis of variance of 8-week weights of pigs grouped by breed, sex and year resulted in significant differences in 8-week weights of pigs among breeds.

The correlation coefficients between 3-week and 8-week weights indicated that weight at 3 weeks of age is very important in determining 8-week weight. Linear regression of 8-week weight on 3-week weight showed that for each additional pound of pig weight at 3 weeks an increase of approximately 2.4 lb. in 8-week weight may be expected.

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BODY MEASUREMENTS IN RELATION TO BEEF TYPE AND TO CERTAIN CARCASS CHARACTERISTICS [1]

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FOR the past 40–50 years there has been considerable endeavor to describe beef type in terms of linear and non-linear body measurements. Moreover, in studies of this nature, the usual practice has been to calculate certain ratios involving linear measurements and body weight for use in the prediction of future performance. Several indexes of this nature have been reported (Yapp, 1923; Black *et al.*, 1938).

The purpose of the present study was to determine which of 10 body measurements were the most reproducible. It was desired to check not only the measurement error of individual investigators, but also the error which can be expected from investigator to investigator. From this study, the most reproducible measurements were to be used to describe Hereford calves which had come from three different breeding sources. Finally, some ratios were to be calculated involving measurements and body weight as predictors of carcass quality.

Experimental

Calves used in this experiment came from three main sources. The cattle which were measured in 1956 consisted of progeny of outbred and inbred Hereford bulls from a topcross breeding project at the Ohio Agricultural Experiment Station, Wooster. Two inbred lines were under test; Montana Line 1, from the U. S. Range Livestock Experiment Station, Miles City, Montana, and Nebraska Line 1 from the University of Nebraska. Outbred bulls used in this project were selected from a purebred herd of Hereford cattle. Of the remaining two groups, one represented the 1956–1957 progeny of a breeding program at the Ohio Agricultural Experiment Station. The other was a group of outbred calves from Texas which had been used in a feeding experiment. Steers and heifers were present in all three experimental groups, and due allowance for sex was made in subsequent analyses.

All calves were measured prior to slaughter at 15 to 18 months of age. The measurements were taken in duplicate by a single investigator on every animal except those calves used in the repeatability study. In the latter case, each of two investigators took duplicate readings of 10 measure-

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ments on eight steers. These steers were chosen at random from the 1955–1956 topcrossed calves. The actual measuring was performed with a steel tape and a pair of large metal calipers.

Fifty-two of the animals used in this study were slaughtered at the Ohio State University Meats Laboratory. Here one-half of each carcass was separated into bone, fat trim and edible portion. The latter is considered edible meat, as no more than 3/8 inch layer of fat remains on any surface. The weights of these three components were then expressed as percentages of the chilled weight of the carcass side. The remaining 71 animals were slaughtered at commercial packing plants and, hence, they yielded no detailed carcass information. However, rib-eye tracings at the 13th rib were obtained for all animals slaughtered from the three breeding groups. Dressing percentages were calculated from Wooster shipping weights and chilled carcass weights.

The statistical procedures employed in the interpretation of results were simple correlation, analysis of variance and covariance techniques as described by Snedecor (1956).

TABLE 1. MODEL FOR CALCULATIONS

Source	df	MSE
Steers (S)	7	$\sigma_E^2+2\sigma_{SI}^2+4\sigma_S^2$
Investigators (I)	1	$\sigma_E^2+2\sigma_{SI}^2+16\sigma_I^2$
S×I	7	$\sigma_E^2+2\sigma_{SI}^2$
Error (E)	16	σ_E^2
Total	31	

Results

Investigator Differences and Measurement Repeatability. As a preliminary study, eight steers were selected at random from the 1956 calves and 10 body measurements were taken as described above. An analysis of variance was run on these data and the steer, investigator, steer-investigator interaction, and error components were estimated. This was accomplished by equating respective mean squares to their mathematical expectations, substituting and solving for the desired components. The model used is presented in table 1.

Table 2 shows the results of these studies. The intraclass correlation was computed to estimate the repeatability of single readings (Lush, 1930; Hetzer *et al.*, 1950). Of particular interest in this latter table are the relative sizes of the four components for any one measurement. In the cases of heart girth, circumference at navel, height at withers and height at hooks, the steer components are relatively high and the error components small, while both the investigator and the interaction components approach zero. Moreover, these were the measurements showing the highest repeatability.

Length of body, depth of chest and width of hooks also had high

TABLE 2. COMPONENTS OF VARIANCE FOR TEN BODY MEASUREMENTS

Variance component for:	Circumference			Length of body	Width		Depth		Height	
	Heart	Navel	Forearm		Chest	Hooks	Chest	Twist	Withers	Hooks
Steers (S)	34.18	45.50	0.40	28.99	1.25	0.79	5.59	0.25	17.17	23.17
Investigators (I)	0.54	0.20	0.79	0.0	0.0	0.0	0.0	2.62	0.0	0.20
Steers×Investigators (SI)	0.15	0.0	1.45	0.0	0.47	0.03	0.0	0.42	0.0	0.0
Measurement error (E)	1.05	4.88	.38	7.06	1.63	.38	.94	2.31	1.88	.88
Repeatability $=\frac{S}{S+I+SI+E}$	0.95	0.90	0.13	0.80	0.37	0.66	0.86	0.04	0.90	0.96

repeatabilities, although the measurement errors were relatively greater than those of the previously mentioned measurements. The significance of this source of error, however, is easily reduced by increasing the numbers of readings taken on each animal.

The two measurements, circumference of forearm and depth of twist had extremely low steer components. In both cases the other three components are nearly as large or larger. The investigator component of 2.6 obtained in the depth of twist measurements suggests that the two investigators were not, in fact, measuring the same distance. Similarly, the high steer-investigator interaction which was found for circumference of forearm data may be explained by the possibility that each man consistently measured the forearms at different levels. Such a procedure could result in a high interaction component as the steers varied somewhat in their

TABLE 3. MEAN SQUARES CORRECTED FOR BODY WEIGHT

Source	Depth of chest	Width of chest	Heart girth	Circumference at navel	Height at hooks	Height at withers	Length of body
Between lines	0	13.5	13.0	407.5**	296.5**	203.5**	486.5**
Between bulls within lines	5.7	12.0	54.0	51.7	16.3	23.0	47.0
Within bulls	6.1	13.0	20.3	61.4	20.4	21.1	48.9

** Significant at 1% level.

weight and fleshing. On the other hand, this interaction may have been due to an inconsistency of each man to measure and same location on different steers.

Line Differences. Three sources of breeding were present in the 1956 group of cattle measured. It was of interest to compare objectively the differences which existed between calves sired by Montana Line 1, Nebraska Line 1 and outbred bulls. For this purpose, the following seven measurements were used: Length of body, height at hooks, height at withers, depth of body, heart girth, circumference at navel and width of chest. As there was a difference in weight between these three groups of calves, a covariance type analysis was used so that group means could be compared at a constant weight. Further analyses revealed that no age differences existed between calf groups and, therefore, no correction for this factor was necessary.

Two bulls from each of the inbred lines and two outbred bulls were tested during 1955–1956. Therefore, it also seemed desirable to study differences between bulls within lines. This would indicate whether or not observed line differences were due to unfortunate sampling of the bulls within each line. This problem was solved by a study of bull variances and covariances irrespective of lines. From both the bull and the line analyses it was possible to construct the table of mean squares corrected for body weight, table 3. In table 4 are recorded line means and their standard errors for the seven measurements studied.

TABLE 4. LINE MEANS [a] AND STANDARD ERRORS CORRECTED FOR BODY WEIGHT

Line	Depth of chest	Width of chest	Heart girth	Circumference at navel	Height at hooks	Height at withers	Length of body
Montana	61.4±0.3	43.8±0.6	177.5±0.6	204.6 ±1.0	118.8±0.6	113.2±0.6	148.8±0.9
Nebraska	61.5± .3	44.8± .6	178.2± .6	206.20±1.0	116.2± .6	111.4± .6	145.0± .9
Outbred	61.6± .4	44.8± .9	178.6± .8	210.8 ±1.3	113.6± .8	108.8± .8	142.3±1.2

[a] In centimeters.

The three variables, depth of chest, width of chest and heart girth showed no significant differences. However, the remaining four measurements, circumference at navel, height at hooks, height at withers and body length had highly significant line mean squares. Moreover, in these four cases, the variability of bulls within lines was very similar to within bull variation. It was therefore concluded that these significant mean squares were due to true line effects.

An inspection of the table of means reveals that Montana, top-crossed calves tended to be taller, longer and less paunchy than either the Nebraska or the outbred calves. In all cases, Nebraska cattle were intermediate and the outbreds were generally the shortest, lowest set and most paunchy animals. Moreover, this study suggests that length, height and circumference at navel are valuable measurements for describing type in beef cattle.

Ratio Study. In 1956, numerous ratios involving the above mentioned measurements and body weight were calculated and correlated with dressing percentage, area of rib eye and percentage edible portion. The two most promising ratios proved to be wt./ht. and wt./length. As a continuation of this study, more measurements were obtained in 1957 on the two additional cattle groups mentioned in the previous section. Correlations of the above two ratios were calculated on a within group and sex basis and the results of these calculations are given in table 5.

TABLE 5. CORRELATIONS OF RATIOS WITH SOME CARCASS CHARACTERISTICS

	Steers			Heifers		
Variable	Dressing percentage	Area of rib eye	Edible portion	Dressing percentage	Area of rib eye	Edible portion
$\frac{\text{Weight}}{\text{Height}}$	0.37**	0.52**	—.45*	0.14	0.39**	—.52*
$\frac{\text{Weight}}{\text{Length}}$	.37**	.51**	—.43*	.19	.45**	—.48*
Total number animals studied	59	57	30	64	64	22

* Significant at 5% level.
** Significant at 1% level.

Both ratios are highly significantly correlated with area of rib eye. Moreover, these correlations seem to be high enough to be of use in selection programs. The highly significant positive correlations of these ratios with dressing percentage in the case of steers and the significant negative correlations with the percentage edible portion in steers and heifers must be due, primarily, to a positive correlation of the ratios with carcass fat. It is well known that the percentage of carcass fat is positively related to dressing percentage and, certainly, the percentage edible portion is negatively correlated with carcass fatty tissue.

These results appear conflicting. On the one hand, ratios involving live body measurements were positively correlated with the area of rib eye, a supposed index of carcass muscling, and on the other hand, these same ratios were negatively correlated with edible portion percentage.

TABLE 6. CORRELATIONS BETWEEN BODY RATIOS, EDIBLE PORTION/BONE, AREA OF RIB EYE AND FAT TRIM[a]

Sex	r_{12}	r_{13}	r_{14}	r_{15}	r_{45}	$r_{14.5}$
Steers	.10	.23	.24	.63**	.45*	−.06
Heifers	.39	.39	.48	.13	.62**	.52*

[a] Edible portion/bone=1; area rib eye=2; wt./length=3; wt./ht.=4; fat trim=5.
* Significant at 5% level.
** Significant at 1% level.

In order to resolve this difficulty and in view of the work of Hankins *et al.* (1943), a new ratio, edible portion/bone (1) was calculated and correlated with area of rib eye (2), wt./length (3), wt./ht. (4), and the percent fat trim in the carcass (5). Moreover, wt./ht. was also correlated with fat trim so that the partial correlation $r_{14.5}$ could be calculated. Again, steers and heifers were kept separate and the results of these analyses are given in table 6.

Definite sex differences are noticeable in these data. However, it is not clear whether or not this variation in performance is due to different levels of carcass fat or to sampling error. The most interesting correlation, $r_{14.5}$, is virtually zero for steers and significantly positive for heifers. These results suggest that it may be possible to select positively for the carcass index, edible portion/bone, by means of the ratios wt./ht. and wt./length.

These studies led to the conclusion that, for breeding work, the ratio of edible portion to bone may be a more satisfactory carcass index than percent edible portion. However, r_{15} is high in the steer data and this indicates that the edible portion to bone ratio should be corrected to a standard fat percentage so that ratios from carcasses of varying fat trim would be directly comparable. Linear regressions of the edible portion to bone ratio on fat trim were run on a within group basis and the results

are shown in table 7. It is evident that the slope of the regression line, b, tends to decrease as the average percent fat trim in the group rises. The negative coefficient of —.079, obtained for heifers with an average of 21% fat trim, is probably due to the low variance in fat trim in this particular sample. Table 7 supports the idea that the influence of fat trim on the weight of edible portion decreases with increasing fat trim.

In view of these findings, a log curve was fitted to the data of table 7. The equation was of the form: Edible portion to bone ratio = b Log (fat trim) + C. In this case, b = 1.6061 and C = 2.6333. Positive devia-

TABLE 7. INTRA-GROUP REGRESSION OF EDIBLE PORTION/BONE ON FAT TRIM [a]

Sex	Number	Average fat trim, %	Variance of fat trim, %	b_{15}
Steers	18	14.2	5.21	+.0760
	20	15.9	2.90	+.0701
	12	16.7	3.67	+.0406
Heifers	14	17.7	5.16	+.0315
	12	21.0	1.99	—.0786

[a] Edible portion/bone=1; fat trim=5.

tions from this regression line are considered to indicate better than average carcasses and vice versa.

This equation may be used for calculating correction factors for different levels of fat trim. For example, if it is desired to standardize carcasses to 15% fat trim, let the ith carcass have a fat trim percentage (F.T.i), and an edible portion to bone ratio, E.P.i/B, then the corrected ratio will be:

$$\frac{E.P.i'}{B} = \frac{E.P.i}{B} + (1.6061 \text{ Log } 15 - 1.6061 \text{ Log } (F.T.i)) = \frac{E.P.i}{B} + 1.6061 \text{ Log } \frac{(15)}{F.T.i}$$

Summary

Ten linear body measurements were taken in duplicate on eight Hereford steers by two investigators. The components of variance due to steers, investigators, steer and investigator interaction and measurement error were estimated and compared. Results of this study indicated that heart girth, circumference at navel, length of body, width of chest, depth of chest and height at hooks and withers had the highest steer components and the lowest investigator, interaction and error components. After this study, additional cattle were measured.

Significant differences in circumference at navel, height at hooks, height at withers and body length were found between calves sired by bulls from different lines of breeding when compared on a constant weight basis.

Ratios of weight to height and weight to length were calculated and correlated with dressing percentage, area of rib eye and edible portion. In both steer and heifer data, the two ratios were positively correlated with area of rib eye and negatively correlated with edible portion. These ratios were positively correlated with dressing percentage in steers but not in heifers.

Percent edible portion is highly influenced by the amount of fat trim in the carcass. Therefore, a ratio of edible portion to bone was calculated and correlated with the weight to height and weight to length ratios. The edible portion to bone ratio is also influenced by the degree of fatness of the carcass. A formula for correcting the edible portion to bone ratio to a constant fat trim percentage is presented.

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AN EVALUATION OF THE ANTIPYRINE DILUTION TECHNIQUE FOR THE DETERMINATION OF TOTAL BODY WATER IN RUMINANTS [1, 4]

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BODY weight changes are employed as the criteria of response in most feeding experiments with ruminants. However, the tissue gained or lost may not be of the same chemical composition; therefore, the same increment in weight resulting from a different experimental treatment does not necessarily reflect equivalent effects. An *in vivo* technique for estimating body composition would be of immense value in experiments where energy gain or loss would be a more significant measure of response than body weight change.

There has accumulated considerable evidence supporting the generalization that the percentages of water, protein and ash approach constancy in the fat free body of animals of the same age and species. Reid *et al.* (1955) have compiled the available data on the composition of the bovine body. Their analysis adequately demonstrates that the entire composition of an animal body can be reasonably resolved if either the fat content or the water content can be determined.

To date the most used test solute for the determination of total body water has been antipyrine (1, 5-dimethyl-2-phenyl-3 pyrazolone). Direct evidence that the antipyrine dilution technique provides a valid estimate of total body water was first obtained by Soberman (1950) using dogs, rabbits, and monkeys. In farm animals Kraybill *et al.* (1951) adapted the antipyrine technique of determining total body water to cattle. Since the paper by Kraybill *et al.* (1951) others have appeared on cattle by MacFadden and Richards (1956), Wellington *et al.* (1956), Reid (1956), Swanson and Neathery (1956), and Klosterman *et al.* (1956); and on sheep by Dumont (1955) and by Hansard and Lyke (1956).

This paper presents the results of several attempts to utilize the antipyrine dilution technique for the determination of total body water in the ruminant. The effects of varying periods of water deprivation on total body water and the antipyrine concentration in the ruminal fluid were

[1] This paper is part of a thesis submitted by the senior author to the Graduate School, University of California, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Nutrition.

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also investigated. The envisioned purpose was to resolve subsequently the entire body composition.

Experimental Procedure and Results

Four Hereford steers from the University herd were used in a preliminary trial to become familiar with the problems involved in handling the animals and to obtain practice with the analytical procedure. From this preliminary work the procedure and technique utilized was essentially that of Kraybill *et al.* (1951) as adapted from the work at Soberman *et al.* (1949). Serum samples were analyzed for antipyrine by the precipitation method of Brodie *et al.* (1949). A standard solution containing a known amount of antipyrine was included with each set of samples. The serum levels of antipyrine were plotted semi-logarithmically against time. The extrapolation to zero time was made by the method of least squares. Determinations of serum moisture were made on serum samples from each animal. Body water was calculated by the expression:

$$\% \text{ body water} = 100 \times \frac{\text{mg antipyrine injected}}{\text{serum } H_2O \text{ antipyrine (mg/liter) x weight (kg)}}$$

Unless otherwise specified all animals were fasted for 24 hours and water was withheld for 12 hours preceding the injection of antipyrine. Each animal was weighed immediately prior to injection and immediately after the last blood sample was taken. The mean weight was used for the calculation of percent body water.

Thirty-six grade Hereford steers were the subjects of the first experiment. The steers were on a forage utilization trial (Meyer *et al.*, 1956) conducted in 1955 from July 12 to October 8. Body water was determined on each steer at the beginning and at the end of the trial by the method previously described. All animals were slaughtered at the close of the experiment and the 9–10–11 rib cut removed from the right side as outlined by Hankins and Howe (1946). Determinations of the specific gravity of this rib cut enabled the estimation of percent body fat in each animal by an eqution given by Kraybill *et al.* (1951–52). From the percent body water determined by antipyrine and using the relationship established by Reid *et al.* (1955) between body water and body fat of cattle, a comparison between the two determinations of body fat is possible.

A summary of this trial appears in table 1. There was an unexpected disagreement between the two methods of estimating body fat in view of the results obtained by Kraybill *et al.* (1951, 1951–52). The correlation coefficient (0.24) between the two methods of calculating body fat was not significant. The fact that there was no apparent relationship between percent body water determined by the use of antipyrine at the beginning of the

trial and at the trial's end may be of more significance than the above correlation.

In the spring of 1956, ten purebred yearling Hereford bulls on a feed utilization trial were the subjects of investigation. Body water was determined three times during the trial between March 15 and July 7. The experimental technique was exactly as before except the injection of antipyrine was made through a small flexible plastic tube (electrician spaghetti)

TABLE 1. SUMMARY OF THE RESULTS OF THE 1955 STEER TRIAL TO DETERMINE BODY WATER BY THE ANTIPYRINE DILUTION TECHNIQUE

Steer no.	Initial weight (kg.)	Initial % H_2O by ap.	Initial % fat calculated [a] from % H_2O	Final weight (kg.)	Final % H_2O by ap.	Final % fat calculated [a] from % H_2O	Final % fat by [b] sp. g.	Slaughter grade
303	420	51.9	26.3	468	53.8	24.1	19.6	Good
307	380	52.8	25.3	384	62.0	15.0	21.5	Good
313	428	65.6	10.9	483	63.0	13.8	19.9	Good
323	385	54.7	23.1	468	58.5	18.9	26.6	Good
328	419	[c]	....	470	52.3	25.8	21.0	Good
329	412	57.8	18.7	467	55.3	22.5	22.1	Good
333	388	[c]	....	439	57.0	20.6	19.9	Good
338	365	[c]	....	430	55.8	21.9	21.0	Good
370	376	[c]	....	420	60.0	17.2	18.2	Good
373	390	48.7	29.8	439	61.5	15.5	24.1	Good
376	420	54.8	23.0	471	[c]	....	18.0	Good
379	400	52.6	25.5	435	46.4	32.4	21.8	Good
394	371	54.0	23.9	453	54.5	23.4	24.4	Good
396	353	63.8	13.0	418	[c]	....	20.7	Good
Mean	393	55.7	21.9	446	56.7	20.9	21.3	
310	350	53.5	24.5	358	59.8	17.4	15.0	Commercial
312	417	49.2	29.3	444	66.6	9.8	19.6	Commercial
315	372	60.7	16.4	413	59.6	17.6	14.5	Commercial
340	370	55.1	22.7	345	54.0	23.9	15.3	Commercial
346	397	60.9	16.2	491	62.8	14.1	15.5	Commercial
347	390	61.1	16.0	449	61.4	15.6	14.5	Commercial
349	401	53.8	24.1	432	55.2	22.6	16.9	Commercial
350	387	57.8	18.7	428	61.8	15.2	18.0	Commercial
353	437	62.7	14.2	484	57.2	20.3	18.5	Commercial
356	378	55.5	22.2	433	[c]	....	18.5	Commercial
358	374	52.2	25.9	396	56.0	21.7	20.1	Commercial
360	395	61.5	15.5	453	64.9	11.7	16.9	Commercial
365	380	52.1	26.0	425	50.3	28.0	21.5	Commercial
368	328	[c]	....	380	54.0	23.9	17.7	Commercial
375	355	56.5	21.1	432	66.3	10.2	17.4	Commercial
377	371	52.5	25.6	408	56.2	21.5	16.6	Commercial
381	375	58.0	19.4	425	58.0	19.4	16.9	Commercial
384	360	58.1	19.3	381	52.0	26.1	16.1	Commercial
386	351	57.7	19.8	393	54.5	23.4	17.2	Commercial
393	373	[c]	....	441	51.3	26.9	24.1	Commercial
395	379	53.9	24.0	426	56.5	21.1	19.9	Commercial
Mean	378	56.3	21.1	421	57.9	19.5	17.6	
372	398	64.5	12.2	406	58.5	18.9	7.7	Utility
Grand mean	385	56.5	21.1	430	57.5	20.0	18.8	

[a] Body fat calculated from body water—Reid *et al.* J. Dairy Sci. 38:1344. 1955.

[b] Body fat calculated from specific gravity of 9/10/11 rib cut—Kraybill *et al.*, J. Applied Physiol. 4:575. 1951–52.

[c] Straight line not obtained when data plotted for extrapolation.

TABLE 2. DETERMINATION OF BODY WATER ON BULLS BY THE USE OF ANTIPYRINE

	March 15, 1956				April 12, 1956			June 7, 1956			
Bull no.	Weight, kg.	Estimated slaughter grade	% H_2O by antipyrine	% fat calculated from % H_2O	Weight, kg.	% H_2O by antipyrine	% fat calculated from % H_2O	Weight, kg.	Estimated slaughter grade	% H_2O by antipyrine	% fat calculated from % H_2O
332	406	Top Comm.	57.8	19.9	444	....	....	498	Choice	51.5	27.3
334	395	Low Good	51.2	27.6	426	....	....	491	Low Choice	67.8	8.0
329	442	Good	64.6	11.8	475	64.6	11.8	533	Top Choice	73.2	0.0
326	432	Good	63.0	13.7	464	67.6	8.3	516	Choice	69.4	6.2
348	336	Low Good	65.6	10.7	375	61.1	16.0	433	Choice	58.4	19.2
335	387	Good	56.2	21.8	421	55.6	22.5	467	Choice	....	....
346	374	Low Good	....	....	411	60.9	16.2	469	Choice	56.8	21.0
336	436	Low Good	....	....	480	52.6	26.0	516	Choice	67.7	8.2
342	371	Low Good	64.8	11.6	417	60.0	17.3	443	Choice	64.6	11.8
343	430	Low Good	....	....	474	66.5	9.6	514	Choice	65.6	10.7

Note: Bulls 332 and 334 not injected on April 12. Other blanks due to injection or sampling difficulties caused by uncooperative subject.

inserted 5–6 inches into the jugular vein. This precaution was taken since during previous trial it was recorded, in one instance, that the needle used to inject the antipyrine had slipped from the vein after a particularly violent motion of the steer during injection. The semi-logarithmic plot of the data for this steer deviated markedly from the straight line usually obtained. This same accident may have occurred with several of the animals (see table 1).

The summary of the results of the trial with bulls appears in table 2. There was no consistent decrease in percent body water as might be expected in animals which visibly increased in finish and gained over 2 lb. per day during the feeding period.

Wellington *et al.* (1956), in a paper concerning the use of antipyrine in nutritional studies with cattle, reported a slightly revised procedure for determining antipyrine in blood serum. This revised procedure recommended that the supernatant resulting after the precipitation of the serum protein be filtered through Whatman No. 42 filter paper to remove traces of a flocculent precipitate which failed to drop during centrifugation. This flocculent precipitate interfered with the spectrophotometric analysis. Preliminary work in this laboratory failed to produce a difference between this revised method for the determination of serum antipyrine and the method then in use. However, a trial was designed to check the laboratory technique for the determination of antipyrine as outlined by Wellington *et al.* (1956) against the procedure used in this laboratory, essentially that of Brodie *et al.* (1949).

Eleven Hereford steers were used in this trial. Large blood samples (about 30 ml.) were taken from each steer to provide sufficient serum for the duplicate analysis. The steers were all slaughtered within two days of the antipyrine body water determination and comparisons were made between percent fat as calculated from body water (Reid *et al.*, 1955) and from dressed carcass specific gravity (Kraybill *et al.*, 1951–52).

The agreement between the two methods of determining serum antipyrine was practically absolute. Perhaps the practice of this laboratory to recentrifuge the decanted supernatant accomplished the same thing as the filter method of Wellington *et al.* (1956).

The results of this trial with 11 steers are shown in table 3. The correlation coefficient between the two measures of body fat was 0.47 and only approached significance at the 5% level.

Possibly the only really valid check of a method for determining body water is an entirely unrelated and direct method such as used by Wellington *et al.* (1956). However, the reports of Kraybill *et al.* (1951, 1951–52) would appear to validate the specific gravity method of estimating body fat in cattle as a suitable basis for comparing the results obtained with the antipyrine technique. This type of comparison has been made with other species as well (Keys and Brozek, 1953).

TABLE 3. SUMMARY OF THE RESULTS OF THE 1956 TRIAL TO DETERMINE BODY WATER ON STEERS BY THE USE OF ANTIPYRINE

Steer no.	Weight, kg.	% H_2O by antipyrine	% fat calculated [a] from % water	% fat calculated from sp. gravity [b]	Slaughter grade
80	424	54.4	23.5	22.5	Choice
04	437	47.2	31.5	24.5	Choice
81	416	49.6	28.8	20.0	Choice
21	456	51.1	27.2	20.4	Choice
96	409	56.2	21.5	20.3	Good
22	416	54.3	23.6	22.1	Good
00	415	54.0	23.9	22.9	Good
95	414	54.7	23.1	23.7	Good
24	385	57.5	20.0	20.9	Good
02	483	50.0	28.4	24.5	Good
92	443	58.5	19.9	19.3	Good

[a] Body fat calculated from body water. Reid *et al.* J. Dairy Sci. 38:1344. 1955.
[b] Body fat calculated from dressed carcass specific gravity. Kraybill *et al.* J. Applied Physiol. 4:575. 1951–52.

The variable results obtained using the antipyrine dilution technique to determine total body water in cattle suggested that the technique was not without difficulties for application in specific investigations. However, an effort was made to determine a cause of these erratic results. Assuming that antipyrine is uniformly distributed in the body water of an animal and is metabolized at a constant rate, the most probable source of error might be associated with the relatively large capacity of the gastrointestinal tract of the ruminant.

A trial with sheep was conducted to determine to what extent the length of fast from food and water affected the subsequent determination of body water by the antipyrine dilution technique. Sheep metabolize antipyrine at a somewhat more rapid rate than cattle (Dumont, 1955; Hansard and Lyke, 1956), but the injection of 10 ml. of solution containing 0.3 of a gram of antipyrine per ml. was found to provide a concentration of antipyrine in the serum water which gave values in the required range for sheep weighing about 100 lb. The results in table 4

TABLE 4. THE EFFECT OF DEGREE OF SHRINK ON BODY WATER IN SHEEP AS DETERMINED WITH ANTIPYRINE

	No shrink		12 hr. without water 12 hr. without food		40 hr. without water 52 hr. without food		Weight change	
Sheep no.	Weight, kg.	% water by antipyrine	Weight, kg.	% water by antipyrine	Weight, kg.	% water by antipyrine	From body weight, kg.	From body water, kg.
66	40.1	63.9	37.2	54.9	34.9	45.9	−2.3	−4.4
78	....	....	49.4	52.9	46.3	48.0	−3.1	−3.9

indicate that as the period of water and food deprivation increased, the percent body water decreased. However, in each instance the calculated water loss was greater than the actual weight loss of the animal. It is entirely possible that 40 hours without water would result in a somewhat dehydrated animal. Therefore, another trial was organized in which the sheep were deprived of food for long periods of time but allowed water up to 12 hours before the injection of antipyrine.

Table 5 gives the results of this experiment with eight sheep. Considerable variation occurs in the percent body water of the individual sheep between the two periods of fast. There appeared to be no constancy between

TABLE 5. THE EFFECT OF WITHHOLDING FOOD ON THE DETERMINATION OF BODY WATER BY ANTIPYRINE IN SHEEP

	24 or 36 hours[a]		48 or 60 hours[a]		Weight change	
Sheep no.	Weight, kg.	% water by antipyrine	Weight, kg.	% water by antipyrine	From body weight, kg.	From body water, kg.
66	37.6	60.8	36.7	53.9	−0.9	−3.1
69	42.9	52.3	41.5	54.3	−1.4	+0.1
78	47.4	43.2	46.9	49.4	−0.5	+2.7
87	41.0	35.9	40.4	36.4	−0.6	−0.0
26	34.9	25.5	33.6	30.4	−1.3	+1.3
37	45.4	50.1	44.0	48.4	−1.4	−1.4
38	50.3	46.0	47.6	54.1	−2.7	+2.6
51	58.1	45.2	55.3	46.2	−2.8	−0.7

[a] Sheep 66–87 were fasted 36 and 60 hours, sheep 26–51 were fasted 24 and 48 hours and all sheep allowed water up to 12 hours before injection of antipyrine.

weight loss as calculated from body water and the actual weight loss determined by weighing.

Rumen samples were obtained by stomach tube from the last four sheep listed in table 5. Analysis of the rumen samples for antipyrine permitted a comparison between the concentration of antipyrine in the rumen water with the concentration of antipyrine in the serum water. The determinations of rumen water antipyrine on sheep no. 26 were inconsistent and deviated markedly from the results obtained with the other animals. This sheep metabolized antipyrine at a very rapid rate (about 60% per hour) and the calculated percent body water was quite low indicating a very fat animal (see table 5.). Sheep 26 was judged by handling to be the thinnest sheep of the group. A pathological condition may have been present. For this reason the results obtained from this sheep have been omitted from table 6.

The values given in table 6 are the rumen water concentrations of antipyrine as a percent of the serum water levels of the drug. It is apparent from these results that antipyrine is not distributed in the rumen water in

the same concentration as in the serum water. Reid (1956) found the concentration of antipyrine in the rumen water expressed as a percent of the amount in serum water in cattle increased from 32 to 86% during the sampling period. These values compared with 75 to 162% found in this investigation indicate a possible species difference in this respect. However, the use of a larger number of animals from each species might find individuals within a species showing as much variation.

Discussion

The work of Kraybill *et al.* (1951, 1951–52) and Wellington *et al.* (1956) indicated that body water in ruminants could be estimated with sufficient accuracy by the antipyrine dilution technique to be used as a

TABLE 6. RUMEN CONCENTRATIONS OF ANTIPYRINE AS A PERCENT OF SERUM CONCENTRATIONS

Sampling time, hours after injection		2.5	3.5	4.5	5.0
Sheep number	Period of fast	$100 \times \frac{\text{rumen water conc. of antipyrine}}{\text{serum water conc. of antipyrine}}$			
37	24 hours	70.5	114.0	165.2	183.0
	48 hours	73.8	109.0	145.8	171.2
38	24 hours	81.1	115.4	149.0	171.7
	48 hours	72.7	108.7	134.7	146.3
51	24 hours	72.6	96.1	116.9	132.6
	48 hours	67.0	88.1	111.8	124.1
Mean	24 hours	74.7	108.5	143.7	162.4
	48 hours	71.2	101.9	130.8	147.2

key for the resolution of body composition in cattle. The experiments reported in this paper suggest that body water as estimated by the antipyrine dilution technique is too variable a quantity to have a direct application in nutritional investigations with ruminants as envisioned at the start of this work. This variability in body water as determined by antipyrine is probably not due to one factor alone, but due instead to a combination of uncontrolled and largely uncontrollable circumstances inherent in the application of the antipyrine technique to ruminants.

The conditions of food and water deprivation imposed upon an animal prior to the injection of antipyrine appeared to have considerable effect on antipyrine space. This has been recognized by MacFadden and Richards (1956) and Reid (1956), but Swanson and Neathery (1956) found the average percent antipyrine determined body water was the same in 14 animals whether fasted or not. Cizek (1954) presents data on the amount of gastrointestinal water after varying periods of fast from food and water in laboratory animals. For example, the guinea pig

deprived of food and water for 5 days still had 10% of the total body water in the lumen of the gastrointestinal tract. Swift (1942) found the contents of the gastrointestinal tract of steers on a maintenance ration differed as much as 16 kg. Swift also observed a difference of 19 kg. in the fill of two similarly treated steers after four days of fast. These differences were due to water.

Blaxter and Rook (1956), on the basis of work with cattle, found the error attached to the estimate of total body water determined by the extrapolation procedure with antipyrine to be $\pm 5\%$ of the amount of water determined. This error was considered entirely too large and these workers rejected the use of antipyrine on this basis. Keys and Brozek (1953) reported a variation of $\pm 5.2\%$ of the mean body water in 26 duplicate measurements of body water in human subjects made a week apart with antipyrine. Variations of this magnitude in human subjects where the rate of metabolism of antipyrine is low and the contents of the gastrointestinal tract are comparatively small would possibly be magnified in the ruminant where the opposite conditions exist.

Findings by Dumont (1955) cast considerable doubt on the validity of assuming equal diffusion of antipyrine in the water of all body tissues. The highest ratio of tissue water antipyrine to serum water antipyrine was 0.85 in the muscle of sheep. The ratio was as low as 0.67 in the lungs. Dumont (1957) also found that antipyrine did not disappear from the different tissues at the same rate. Dumont suggested that the precision of the antipyrine method appeared to be a function of the rate of metabolism of the drug. Swanson and Neathery (1956) also reported that either high or low rates of antipyrine metabolism frequently resulted in unreasonable estimates of body water.

The comparison of body fat calculated from antipyrine body water and from a specific gravity method, as made in this study, could not be expected to show complete agreement. The condemnation of the antipyrine technique for determining total body water in ruminants on this basis alone would not be adequate. However, the fact that the correlation coefficients calculated from the comparison of these two measures of body fat were not significant, together with the incongruity of the duplicate and triplicate body water determinations on an individual animal leads to the same conclusion on a sound basis.

Summary

The relationships among the major chemical components of the animal body permit the resolution of body composition if either the water or the fat content can be determined. The determination of total body water by the antipyrine dilution technique seemed to have sufficient merit to warrant a large scale evaluation of this procedure by direct application in nutritional investigations.

Over 110 individual attempts to determine total body water in 57 beef cattle were made. Duplicate determinations of body water were made three months apart in 36 steers. Triplicate determinations were made in 10 bulls at intervals of one and two months. The individual results in most instances where duplicate or triplicate determinations of total body water can be compared for a single animal show more variation than can be considered physiologically normal for the conditions of the trial. There are 45 direct comparisons between body fat calculated from antipyrine body water and from specific gravity methods. The correlation coefficients between these two measures of body fat were not significant.

Sheep were used in a series of trials to obtain some information concerning the effect of gastrointestinal tract contents on the total body water of the ruminant as estimated with antipyrine. The difference in antipyrine space obtained from the same animal after varying periods of water and food deprivation was considerable. Rumen samples taken by stomach tube during one of the trials found the concentration of antipyrine in the rumen water as a percent of the concentration of antipyrine in the serum water varying from 70 (2.5 hours post-injection) to as much as 180 (5.0 hours post-injection).

The results of these trials are interpreted as indicating that the antipyrine dilution technique as utilized in this investigation gave values for total body water which were too variable to be successfully employed in resolving the body composition of the ruminant.

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THE RATE OF FAT DEPOSITION IN FOUR LINES OF SWINE [1]

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IT is generally agreed that the development of superior strains of meat-type swine would be facilitated by an accurate method of determining the composition of the live animal. The body relationships are such that if the total water, fat, or nitrogen content could be determined, the total body composition could be estimated with a reasonable degree of accuracy. Following the demonstration of a relationship of backfat thickness to the percentage of ether extract in the carcass (Hankins and Ellis, 1934) considerable attention has been given to the patterns of backfat deposition, growth rate and carcass quality. Aunan and Winters (1952) developed a method of backfat measurement which involved the removal of a core of fat from the carcass. Hazel and Kline (1952) published a technique which is widely used in which backfat thickness is determined by the introduction of a probe through the skin to the connective tissue barrier of the muscular structures. DePape and Whatley (1956) found that the average of six probes on the live, market weight hog was more highly correlated with carcass quality than was backfat thickness measured on the carcass. Following the development of an instrument by Andrews and Whaley (1954) in which fat and muscle are distinguished by measuring their electroconductivity, it was decided to investigate the rate of fat deposition of three inbred lines of swine and one group of crossbred pigs.

Experimental Procedure

This experiment was conducted during the spring and summer of 1954. Ninety-seven pigs were distributed within and between three inbred lines as follows: 37 White King (inbred Chester White), 28 Yorkshire, 13 Boilermakers (inbred Chester White), and 19 White King-Boilermaker crosses. The pigs were allotted to one acre of alfalfa ladino pasture by lines, both sexes from three or more litters being represented in each lot. Two lots were used in each line. The pigs were produced on the Swine Breeding Unit of Purdue Livestock Experimental Farm, and represented the results of the inbreeding work which has been in progress since 1940. The White King line had an average inbreeding coefficient of 51. The inbreeding coefficient for the Boilermakers was 33, and that for the Yorkshire was 25. The White King-Boilermaker cross represented the first cross between the inbred lines.

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TABLE 1. COMPARATIVE GROWTH OF SEVERAL LINES OF SWINE UNDER PASTURE CONDITIONS DURING SUMMER OF 1954 (56 DAYS)

Items	White King		Yorkshire		Boilermaker		White King-Boilermaker	
	Barrows	Gilts	Barrows	Gilts	Barrows	Gilts	Barrows	Gilts
Number of pigs	13	24	13	15	6	7	13	6
Av. initial weight, lb.	103.9	96.1	103.7	97.9	100.7	100.3	101.7	98.0
Av. final weight, lb.	207.9	181.7	159.6	160.3	163.0	156.7	190.2	186.5
Av. total gain/pig, lb.	104.0	89.7	55.9	63.3	62.3	56.4	88.5	88.5
Av. gain per day, lb.	1.86	1.60	1.00	1.11	1.11	1.00	1.58	1.58

Individual weights, average feed consumption and average feed efficiency were obtained at weekly intervals. Since this was a study of comparative growth between breed and sex, and since it is generally agreed that fattening becomes an important factor at approximately 100 lb., the growth period was divided into two periods consisting of 8 weeks prior to the attainment of an average weight of 100 lb., and 8 weeks thereafter. Results reported herein are for the period from 100 lb. to market weight of approximately 200 lb. A complete mixed ration was self-fed until the pigs averaged 75 lb. in weight. The ration was composed of ground yellow corn 64%, ground oats 15%, Supplement V 19%, and mineral mixture, 2%. Purdue Supplement V had the following composition: 40% soybean oil meal, 25% meat and bone scraps, 25% fish meal, and 10% cottonseed meal. Mineral mixture was composed of 40% ground limestone, 40% steamed bone meal and 20% iodized salt. From an average weight of 75 lb. to market weight the pigs were self-fed yellow shelled corn, Supplement V, and the 40–40–20 mineral mixture.

When each pig reached a weight of 100 lb., and at each 25 lb. increment thereafter, backfat measurements were made with the Lean Meter. Two measurements were made immediately posterior to the shoulders, two at the mid-lumbar region, and two over the ham, approximately 1½ in.

TABLE 2. COMPARATIVE FEED CONSUMPTION AND FEED EFFICIENCY OF SEVERAL LINES OF SWINE DURING A 56-DAY PERIOD IN THE SUMMER OF 1954

Items	White King	Yorkshire	Boilermaker	White King-Boilermaker
Number of pigs	37	28	13	19
Av. initial wt., lb.	98.9	100.6	100.5	100.5
Av. final wt., lb.	190.9	160.0	159.6	189.0
Av. daily gain, lb.	1.69	1.06	1.05	1.60
Average daily feed consumed, lb.	5.43	3.68	3.61	5.44
Feed per pound of gain, lb.	3.21	3.47	3.42	3.45

TABLE 3. SQUARE OF CORRELATION COEFFICIENTS (R^2)

Line	Sex	Variables		
		X_1X_9	X_1X_3	X_1 alone
White King	Barrows	0.837	0.826	0.823
White King	Gilts	0.831	0.821	0.800
WK x Bm	Barrows	0.780	0.764	0.748
WK x Bm	Gilts	0.779	0.761	0.731
Yorkshire	Barrows	0.679	0.653	0.619
Yorkshire	Gilts	0.670	0.654	0.616
Boilermaker	Barrows	0.792	0.754	0.737

anterior to the tail setting. The site of each of the six probes was approximately 1½ in. lateral to the midline of the animal.

At the termination of each trial the barrows were taken off feed for 12 hours, weighed and trucked to slaughter. Gilts were returned to the breeding herd. Hot carcass weight was taken immediately after the slaughter and dressing process, and the chilled carcass weight was taken after the carcass had cooled 12 to 14 hours. Backfat measurements were taken at the conventional sites, i.e., first rib, last rib, and last lumbar vertebra.

Results and Discussion

The results summarizing by lines and sex the comparative growth rates are presented in table 1. Feed consumption and feed conversion figures are shown in table 2. In all lines, the average backfat of the barrows exceeded that of the gilts at 200 lb. weight.

The White King barrows and gilts gained at the average rate of 1.86 lb. and 1.60 lb. per day, respectively, for the eight-week period subsequent to the time the pigs averaged 100 lb. During the same period the Boilermaker barrows and gilts gained an average of 1.11 and 1.00 lb. per day,

TABLE 4. BODYWEIGHT AND BACKFAT THICKNESS IN FOUR LINES OF SWINE

Body weight	Backfat			
	White King	Yorkshire	Boilermaker	White King-Boilermaker
lb.	in.	in.	in.	in.
100	0.98	0.82	1.22	1.12
125	1.14	1.00	1.28	1.21
150	1.32	1.11	1.40	1.36
175	1.49	1.20	1.55	1.50
200	1.64	1.34	1.71[a]	1.62

[a] Barrows only.

respectively. The White King-Boilermaker barrows and gilts both gained at the average rate of 1.58 lb. per day, while the Yorkshire gilts and barrows gained an average of 1.11 lb. and 1.00 lb. per day, respectively.

Analysis of the data for all weights, sex, lines and sites of measurements (tables 4 and 5) showed that the difference in average backfat due to weight was significant at the 1% level; the difference due to sex approached significance at the 5% level; the difference between lines and difference at various sites of measurement were each significant at the 1% level. These differences indicated it would be better to keep lines and sex separate for prediction purposes rather than pooling over these factors. To obtain a prediction equation for thickness of backfat for each of the lines studied,

TABLE 5. BACKFAT THICKNESS OF BARROWS AND GILTS AT 200 LB. LIVEWEIGHT

Sex	White King	Yorkshire	Boilermaker	White King-Boilermaker
	in.	in.	in.	in.
Barrows	1.71	1.42	1.71	1.64
Gilts [a]	1.60	1.28	....	1.59

[a] Trial concluded before Boilermaker gilts reached 200 lb. liveweight.

regression coefficients were determined for equations of the following form:

$$y=a+b_1+b_2 X_2+\ldots\ldots\ldots b_9 X_9$$ [1]

where y = thickness of backfat measured in millimeters; X_1 = weight in pounds; X_2=age in days; X_3=rate of gain in pounds per day; $X_4 = X_1 X_2$; $X_5 = X^2_1$; $X_6 = X^3_1$; $X_7 = X_1 X^2_2$; $X_8 = X^2_1 X_2$; $X_9 = X^2_1 X^2_2$. The coefficients for the nine variables were determined for each sex of the White King line, White King-Boilermaker cross, Yorkshire line and for the barrows of the Boilermaker line.

The square of the multiple correlation coefficient (table 3) based upon 27 degrees of freedom indicated that approximately 84% of the variation in backfat in barrows of the White King line could be accounted for by the above nine variables. When only X_1, X_2 and X_3 were used, approximately 83% of the variation in backfat could be attributed to weight, age and rate of gain. The square of the correlation between backfat and weight for White King barrows ($r^2 = 0.823$) indicates that 82% of the variance in backfat thickness in White King barrows was attributable to weight alone. Therefore, the backfat prediction equations where weight, age and rate of gain were considered became:

$$y=0.136+0.1748X_1+0.2296X_2+0.0166X_3 \text{ for barrows}$$
$$y=0.142+0.1641X_1+0.0110X_2+0.0260X_3 \text{ for gilts}$$

[1] Computations on the prediction equation were made on the Datatron in the Statistical and Computing Laboratory.

For gilts the resultant r^2 was 0.800 when the correlation of backfat thickness on weight alone was computed. When regression coefficients for X_1 through X_3 were computed the R^2 was 0.831, again indicating that the greatest portion of backfat thickness was attributable to weight alone. Since age and weight were highly correlated ($r = 0.89$ for White King barrows and 0.92 for Yorkshire barrows) and R^2 for weight was larger (R^2 for weight, 0.82 vs. R^2 for age, 0.68 for White King barrows, and 0.62 vs. 0.53 for Yorkshire barrows) than R^2 for age, the authors felt that using a prediction equation with weight only was justified.

Similar computations were made for the other lines studied. An R^2 of 0.679 based on 18 degrees of freedom was obtained for Yorkshire barrows when all nine terms were used, 0.653 when X_1, X_2 and X_3 were used, and 0.619 when X_1 or weight alone was used. For White King-Boilermaker pigs, a comparison of R^2 when weight alone was used with those obtained when X_1 through X_9 and X_1 through X_3 were used showed 78% of the variation was attributable to X_1 through X_9, while 75% was attributable to X_1 only. Since such a small contribution to the variation in backfat thickness was due to any of the variables studied other than weight, the single regression curves were calculated.

Such equations for the various lines took the following form:

White King barrows	$y = 4.02 + 0.1976\,X$
White King gilts	$y = 3.31 + 0.1889\,X$
Yorkshire barrows	$y = 9.45 + 0.1263\,X$
Yorkshire gilts	$y = 10.25 + 0.1041\,X$
White King-Boilermaker barrows	$y = 12.60 + 0.1540\,X$
White King-Boilermaker gilts	$y = 13.50 + 0.1440\,X$
Boilermaker barrows	$y = 11.80 + 0.1605\,X$
Boilermaker gilts	none

where X = weight in pounds and y = backfat in millimeters at weight X.

It has been known for a number of years that the relative rate of fat versus muscle deposition in swine differs considerably depending on age and size of the animal. McMeekan (1940) showed that in the new born pig the carcass was composed of 30% muscle and 5% fat. At the age of 20 weeks and approximately 115 lb. in live weight the carcass was composed of 44% muscle and 32% fat, and at 28 weeks and 220 lb. live weight the carcass was composed of 39% muscle and 43% fat. A graph in which fat at various ages as percent of fat at birth is plotted against age forms an exponential type curve. Since the fat is being laid down over an ever increasing body area it was thought that in the present work it might be reasonable to assume a straight line relationship of backfat versus body weight. An examination of the prediction equation for backfat thickness and factors considered in their development indicates the assumption was reasonable.

Hetzer *et al.* (1956) in measurement of backfat on 140 live hogs consisting of 45 boars, 30 barrows and 65 gilts found that although the thickness of backfat in barrows was intermediate between that in boars and gilts at 150 lb. live weight, the barrows laid on fat significantly faster, so that above 200 lb. they exceed both the boars and gilts. They also suggested that when live measurements were made at weights between 175 and 225 lb. they were as accurate as carcass backfat measurements. Their regression equations are:

$$y = 0.28 + 0.0057X \text{ for barrows}$$
$$y = 0.57 + 0.0042X \text{ for gilts}$$

where y = backfat in inches and x = weight in pounds.

These equations indicate that for each 10-lb. increase in body weight the thickness of backfat would be expected to increase by an average of 0.057 in. and 0.042 in. for barrows and gilts, respectively. A transformation

TABLE 6. BODYWEIGHT AND BACKFAT THICKNESS IN SWINE[a]

Body weight	Backfat thickness			
	Shoulder	Loin	Ham	Average
lb.	in.	in.	in.	in.
100	1.19	0.83	0.94	0.98
125	1.38	0.97	1.09	1.15
150	1.53	1.10	1.23	1.28
175	1.69	1.23	1.37	1.42
200	1.86	1.36	1.48	1.56

[a] Averages of four lines.

of the regression equations for Yorkshire barrows and gilts in this work to units comparable to those of Hetzer *et al.* (1956) gives the following:

$$y = 0.37 + 0.0051X \text{ for barrows}$$
$$y = 0.40 + 0.0041X \text{ for gilts}$$

These equations show an increase of 0.051 in. and 0.041 in. of backfat, respectively, for each 10-lb. increase in weight, which is in good agreement with Hetzer's work. Equations for backfat of barrows and gilts of the other breeds studied indicate a greater increase in fat thickness per unit increase in weight than was observed in hogs studied by Hetzer *et al.* or in the Yorkshire hogs of this study.

It should be pointed out that if a study were to be made on the regression of backfat, on age and previous pattern of growth in pigs of single weight classes more individual pigs than were in this study would be required for each weight class and a more clear-cut weight classification would be desired to allow a precise estimate of the regression coefficient within the various weight classes.

Summary

In a comparative growth study observations on rate of gain, feed efficiency and fat deposition were made on 97 animals, representing four different lines of breeding. More than 3,000 individual backfat measurements are included in the study.

An analysis of variance of the data for all weights, sex, lines, and sites of measurement showed that the difference in backfat thickness due to weight was highly significant ($P < 0.01$). There were highly significant differences in average backfat between lines ($P < 0.01$). The Yorkshire swine of all weights had significantly less backfat than any of the other lines. At 100 lb., backfat of the pigs from the White King-Boilermaker cross was intermediate between the parent lines while at 200 lb. the backfat of the crosses was no greater than that of White Kings (table 4).

In each line studied thickness of fat was greatest over the shoulder and least at the loin site. At 200 lb. liveweight, in each of three lines, gilts had less backfat than barrows (experiment concluded before Boilermaker gilts reached 200 lb.) Equations were evolved for predicting average backfat thickness for each line and sex, using weight as the independent variable.

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COMPARATIVE EFFECTS OF UREA, URAMITE, BIURET SOYBEAN PROTEIN, AND CREATINE ON DIGESTION AND NITROGEN METABOLISM IN LAMBS [1, 2]

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ALTHOUGH the use of urea as a protein substitute in ruminant nutrition has become a common practice, its use as the major or sole source of nitrogen is limited by inefficient utilization and the danger of ammonia toxicity (Mills *et al.*, 1942; Pearson and Smith, 1943). The entrance of large quantities of urea into the rumen and its subsequent rapid hydrolysis results in the release of ammonia at a rate which does not permit efficient use of the nitrogen by rumen microorganisms for the synthesis of cellular protein. Ammonia not utilized by the rumen flora is rapidly absorbed into the blood stream where it may reach toxic levels (Dinning *et al.*, 1948).

These adverse affects of urea might be partially eliminated through the use of more slowly hydrolyzed, non-toxic, non-protein nitrogen (NPN) compounds. From this standpoint, combinations of urea and Uramite, a urea-formaldehyde condensation compound of low solubility described by Kralovec and Morgan (1954), seemed worthy of study. Another compound of interest is biuret, which was shown by Repp *et al.* (1955) to be less toxic than urea. Gaither *et al.* (1955) demonstrated that lambs retained the nitrogen from biuret as well as they did that from urea. On the other hand, Hatfield *et al.* (1955) found that biuret nitrogen was not retained by steers as well as urea nitrogen. These results suggested the desirability of obtaining additional information on this material. Creatine nitrogen was shown by Belasco (1954) to be readily available to rumen microorganism *in vitro,* but information on its utilization by the ruminant is needed. In the present work digestion and nitrogen metabolism trials were conducted with lambs to compare Uramite, crude biuret, biuret, and creatine with purified soybean protein and urea as sources of nitrogen.

Experimental Procedure

Three standard nitrogen metabolism and digestion trials were conducted with grade Hampshire wether lambs that averaged 65 lb. in weight. Before

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 578 from the Departments of Animal Husbandry and of Agricultural Biochemistry and Nutrition.

[2] The "Uramite" was generously supplied by E. W. Kolterman of E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware. Choline chloride, thiamin hydrochloride, riboflavin and alpha tocopherol were supplied through the courtesy of L. Michaud, Merck, Sharp and Dohme, Rahway, New Jersey. The feeding urea, biuret, crude biuret, and creatine were generously supplied by D. F. Green, Nitrogen Division, Allied Chemical and Dye Corporation, New York.

being placed in the metabolism crates, the lambs were treated with phenothiazine and lead arsenate.

The metabolism crates were similar to the one described by Briggs *et al.* (1949) with the modifications described below. The wood floor was replaced with one of expanded steel. The round, galvanized metal urine collection funnel was replaced with one made of stainless steel constructed so that the collection area was a square with dimensions equal to the width of the crate. A removable aluminum screen was fitted into the top of the collection funnel. Separation of the feed box from the urine collection funnel by about 3 in. of the expanded steel floor prevented feed from getting into the urine. These modifications resulted in a marked improvement in collection efficiency and greatly reduced contamination.

Each trial was preceded by an adjustment period during which the feeding level to be used in the subsequent experimental period was established. This level was equal to the maximum amount of feed which would be consumed completely by all lambs. This amount was sufficient to satisfy maintenance requirements, and usually permitted a small gain in weight. The adjustment period was followed by a preliminary period during which the experimental rations were fed at the previously established level. The collection period began after all lambs had consistently consumed their complete rations each day for at least 10 consecutive days.

Feces and urine were collected over a 10-day period. Samples equal to 10% of the daily excreta were taken for analyses. Fecal decomposition was minimized by the daily addition of thymol crystals and by keeping the samples under refrigeration. To minimize loss of urinary nitrogen, 15 ml. of 0.38M copper sulfate in 4N sulfuric acid were placed in the urine collection bottles each night. Samples were kept under refrigeration until analyzed.

The composition of the semi-purified, urea-basal rations used in this investigation are presented in table 1. Purified soybean protein supplied 15% and urea alone or in combination with experimental NPN materials contributed 67% of the total ration nitrogen. In each trial the urea-basal ration was fed to a control group of lambs. All rations contained approximately 1.5% nitrogen and 25% crude fiber.

In preparing the experimental rations, the NPN compound under study was added to the control ration at the expense of urea. When the quantity of the experimental material required to supply the desired level of nitrogen exceeded the amount of urea, further substitution was made at the expense of dextrose. This procedure was followed in order to make all rations as nearly iso-caloric and iso-nitrogenous as possible.

All ingredients for each ration, except the molasses and wheat straw, were combined into a concentrate mixture. This mixture, the wheat straw, and the molasses for each feeding were thoroughly mixed by hand immediately before feeding. The rations were fed in equal amounts twice daily. The lambs had free access to water between feedings. During each trial,

appropriate samples of the three ration moieties were taken for proximate analyses which were made according to A.O.A.C. methods (1950).

In trial I, Uramite was studied alone and in combination with urea. Enough Uramite, which contained 36.9% of nitrogen on a dry-matter basis, was substituted for urea so that it provided 2, 4, 8, 16, and 32% of the

TABLE 1. AVERAGE DAILY AMOUNT AND PROXIMATE COMPOSITION OF THE UREA-BASAL RATIONS

Item	Trial no.		
	I	II	III
Ration constituents, grams			
Wheat straw	425.0	440.0	440.0
Cane molasses (78% solids)	167.0	170.0	170.0
Purified soybean protein[a]	11.9	13.2	13.2
Urea[b]	16.6	18.4	18.4
Cornstarch	69.0	76.1	76.1
Dextrose	29.6	32.3	32.3
Corn oil	23.0	29.4	29.4
Wheat germ oil	3.3	.....	.....
Mineral mixture[c]	24.7	27.3	27.3
Vitamin A-D fish oil[d]	2.2	2.6	2.6
Choline chloride	0.7	0.7	0.7
Total	773.0	810.0	810.0
Thiamine HCl	..... 1.8 mg. per day		
Riboflavin	..... 1.8 mg. per day		
Alpha tocopherol	.....	8.0 mg. per day	
Composition, dry matter basis, %			
Organic matter	92.91	92.73	93.74
Protein (N x 6.25)	9.31	9.38	9.79
Ether extract	4.95	5.18	5.00
Crude fiber	26.45	24.82	24.41
Nitrogen-free extract	52.20	53.35	54.54

[a] Drackett assay protein C-1 obtained from the Drackett Products Co., Cincinnati 32, Ohio.
[b] Arcadian feeding grade urea, Nitrogen Division, Allied Chemical & Dye Corp., New York, contained 42.8% nitrogen on dry matter basis.
[c] Described in Thomas *et al.* (1951).
[d] "Nopco XX", National Oil Products Co., Harrison, N. J. Guaranteed to contain no less than 2,250 U.S.P. units of vitamin A and 3,000 I.C. units of vitamin D per gram.

supplementary nitrogen. Crude biuret as the only source of supplementary nitrogen and a combination of crude biuret and urea on a nitrogen equivalent basis were compared with urea in Trial II. In Trial III urea was compared with purified soybean protein, pure biuret, and a combination of creatine and urea.

In this paper the term "percent of absorbed nitrogen retained" is equivalent to the term "biological value" as calculated by the Thomas-Mitchell equation. The former term is used because it is considered to be more applicable to ruminant rations containing high percentages of NPN. Values for

metabolic fecal nitrogen (0.435 gm. per 100 gm. of dry matter intake) and endogenous urinary nitrogen (0.034 gm. per kilogram of body weight) were obtained with eight lambs fed the basal ration described in table 1 without urea and soybean protein. This ration contained 0.26% nitrogen and was fed to the lambs during a 25-day adjustment and preliminary period and then for two consecutive 5-day collection periods.

Results and Discussion

Trial I. Known characteristics of Uramite, as reported by Kralovec and Morgan (1954), suggested that it would not be rapidly hydrolyzed in the rumen. It was hoped, however, that the rate of hydrolysis would be such

TABLE 2. TRIAL I. INFLUENCE OF URAMITE UPON NUTRIENT DIGESTIBILITY AND NITROGEN RETENTION (AVERAGE DAILY BASIS)

	Source of supplemental nitrogen					
Item	100% Urea control	98% Urea 2% Uramite	96% Urea 4% Uramite	92% Urea 8% Uramite	84% Urea 16% Uramite	68% Urea 32% Uramite
Number of lambs	2	2	2	2	2	2
Ration protein (N×6.25)%	9.31	9.19	9.12	9.30	9.30	9.40
Average dry matter intake, gm.	688	688	688	688	688	688
Average dig. coefficients						
Organic matter	62.5	60.6	60.4	60.0	61.4	62.1
Protein (N×6.25) [a]	69.0	66.6	65.2*	64.4*	62.8*	55.8*
Crude fiber [b]	55.3	49.9**	50.9*	52.2*	53.3*	56.9
N-free extract	63.7	63.0	63.0	61.8	63.7	64.6
Nitrogen intake, gm.	10.24	10.10	10.04	10.24	10.24	10.37
Fecal nitrogen, gm.	3.18	3.37	3.49	3.65	3.80	4.59
Urinary nitrogen, gm.	6.90	7.07	6.97	7.45	6.51	6.23
Nitrogen balance, gm.	+0.16	−0.34	−0.42	−0.86	−0.07	−0.45

* $P<.05$.
** $P<.01$.
[a] LSD of $P<.05$, 3.19.
[b] LSD of $P<.05$, 2.57.

that when Uramite was fed with urea, the efficiency of nitrogen utilization would be greater than obtained with urea alone.

The data presented in table 2 indicate that much of the nitrogen in Uramite does not become available and is excreted in the feces. The apparent digestibility of crude protein was significantly reduced when 4, 8, 16, and 32% of the supplemental ration nitrogen was supplied by Uramite.

Uramite at levels of 2 and 4% brought about a highly significant ($P<.01$) reduction in digestibility of crude fiber. At the 8 and 16% levels of Uramite, the depression in crude fiber digestibility was significant ($P<.05$), but not at the 32% level. No explanation for this difference in the digestibility of crude fiber as a function of Uramite level is available.

Trial II. The crude biuret used in this trial was stated to contain 40.0% biuret, 45.5% urea, 6.7% triuret, 6.7% cyanuric acid, and 0.3% water. At

the levels fed, this would be equivalent to supplying 20 and 40% of the supplemental nitrogen in the form of biuret.

In this trial, which is summarized in table 3, nutrient digestibility and nitrogen utilization were not significantly influenced by crude biuret except for the sigificant ($P<.05$) depression in apparent digestibility of crude protein when all supplemental nitrogen was supplied by crude biuret.

TABLE 3. TRIAL II. INFLUENCE OF CRUDE BIURET UPON NUTRIENT DIGESTIBILITY AND NITROGEN UTILIZATION (AVERAGE DAILY BASIS)

Item	Source of supplemental nitrogen		
	100% Urea	50% Urea 50% Crude biuret	100% Crude biuret
Number of lambs	4	4	4
Ration protein (N x 6.25) %	9.38	9.68	9.42
Average dry matter intake, gm.	728	728	728
Average dig. coefficient			
Organic matter	61.3	59.0	57.7
Protein (N x 6.25)	66.7	65.3	61.1*
Crude fiber	50.0	46.0	46.8
N-free extract	63.7	60.3	62.9
Nitrogen intake, gm.	10.86	11.30	10.91
Fecal nitrogen, gm.	3.62	3.94	4.25
Urinary nitrogen, gm.	6.52	6.32	6.28
Nitrogen balance, gm.	+0.72	+1.04	+0.38
Absorbed N retained, %	48.3	50.9	48.0

* $P<.05$.

Trial III. Since the value of biuret as a source of nitrogen in trial II may have been obscured by the presence of urea and other compounds in the biuret mixture, in trial III pure biuret was used as the only source of supplemental nitrogen. In addition, purified soybean protein and creatine were also evaluated as nitrogen sources. No evidence of toxicity was observed when urea or either level of crude biuret was fed and on the basis of the report by Berry *et al.* (1956) manifestations of toxicity would not be expected.

The results of this trial are presented in table 4. Nutrient digestibility was significantly reduced when pure biuret provided all of the supplemental nitrogen. In addition, nitrogen utilization was adversely affected as indicated by the negative nitrogen balance and the highly significant reduction in the percentage of absorbed nitrogen retained. A much greater reduction in nutrient digestion and nitrogen retention was obtained with pure biuret than with the crude biuret in the previous trial. It appears that nitrogen from pure biuret is poorly utilized in lambs. This is not in agreement with the results of Gaither *et al.* (1955) who reported that biuret nitrogen

was retained by lambs as well as urea nitrogen. It should be noted that their lambs had been on a NPN ration for 37 days before collection began, whereas our lambs had only a 10-day preliminary period. Other work in our laboratory, Welch *et al.* (1957) has shown that NPN utilization may be improved with time, and this effect may explain the difference in results.

Soybean protein and a nitrogen equivalent mixture of creatine and urea significantly improved nutrient digestibility and nitrogen utilization over

TABLE 4. TRIAL III. THE EFFECT OF UREA, SOYBEAN PROTEIN, BIURET, AND CREATINE UPON NUTRIENT DIGESTIBILITY AND NITROGEN UTILIZATION (AVERAGE DAILY BASIS)

Item	Least significant difference P<.05	Source of supplemental nitrogen[a]			
		Urea 100%	Soybean protein 100%	Biuret 100%	Urea 50% Creatine 50%
Number of lambs		4	3	2	2
Ration protein (N x 6.25) %		9.79	10.22	10.37	9.82
Average dry matter intake, gm.		725	720	722	727
Average dig. coefficients					
Organic matter	2.3	60.7	62.9*	51.3*	62.6*
Protein (N x 6.25)	3.9	67.4	69.5	62.6*	68.2
Crude fiber	3.2	49.6	53.8*	29.8*	54.6**
N-free extract	5.0	62.4	63.9	56.2*	60.6
Nitrogen intake, gm.		11.38	11.80	11.98	11.42
Fecal nitrogen, gm.		3.71	3.60	4.44	3.62
Urinary nitrogen, gm.		7.24	6.41	8.04	6.29
Nitrogen balance, gm.		+0.43	+1.79	−0.70	+1.51
Absorbed N retained, %		43.6	54.6**	34.2**	53.3*

* P<.05.
** P<.01.
[a] On a dry matter basis, soybean protein contained 14.3; biuret 41.9, and creatine 34.0% nitrogen.

that obtained when urea was the sole source of supplemental nitrogen. The data in table 4 suggest that lambs receiving these two sources of nitrogen performed equally well.

Summary

Digestion and nitrogen metabolism trials were conducted to compare Uramite, a urea-formaldhyde condensation compound; crude biuret; biuret; purified soybean protein; and creatine with urea as sources of nitrogen for lambs. The various compounds studied were included alone or with urea in semi-purified, iso-caloric, and iso-nitrogenous rations to provide 67% of the ration nitrogen.

Nitrogen from Uramite was used inefficiently. A significant decrease in the apparent digestibility of crude protein was obtained when it contributed 4, 8, 16, and 32% of the supplementary non-protein nitrogen. A highly

significant decrease in crude fiber digestibility resulted with the 2 and 4% levels of Uramite, a significant decrease with the 8 and 16% levels but no decrease with the 32% level.

Nitrogen utilization and nutrient digestion were not significantly changed when 50% of the supplemental nitrogen was supplied by crude biuret. Apparent digestibility of protein was significantly depressed when all supplemental nitrogen was supplied by crude biuret. When pure biuret supplied 100% of the supplemental nitrogen instead of urea, nutrient digestion and nitrogen utilization were significantly depressed.

Nutrient digestibility was greater when purified soybean protein or a nitrogen equivalent mixture of urea and creatine replaced urea as the source of supplemental nitrogen. This improvement, however, was significant only in the case of organic matter and crude fiber digestibility. Nitrogen utilization was significantly improved by soybean protein and creatine. None of the materials at the levels studied elicited any signs of toxicity.

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THE EFFECT OF SELENIUM ON REPRODUCTION IN SWINE [1]

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EVIDENCE that selenium can interfere with the normal development of the embryo and that it can produce malformations in the progeny has been reported by Franke and Tully (1936) for the chick, by Rosenfeld and Beath (1947) for sheep, and by Franke and Potter (1936) for the rat. The effect of selenium on reproduction in swine has not been reported. Previous work at this Station (Wahlstrom *et al.*, 1955, 1956) showed the effects of selenium toxicity in growing swine. It was also reported that the organic arsenicals would give protection against selenium poisoning in growing swine.

The studies reported herein were conducted to determine the effect of selenium on reproduction in swine, and the value of arsanilic acid as a selenium poisoning preventive.

Experimental Procedure

Thirty purebred Duroc gilts were allotted to three experimental treatments on the basis of litter and body weight. These pigs were approximately 8 weeks of age and averaged 33 lb. when placed on the experiment. This experiment was initiated on August 6, 1956 and terminated when the sows weaned their second litter of pigs. The basal rations fed are shown in table 1. These rations were self-fed at all times except for about 3 days pre- and post-farrowing when the ration was hand-fed. Selenium was added as sodium selenite to furnish 10 p.p.m. of selenium in the total rations fed to Lots 2 and 3. This level of selenium has been found to be slightly toxic and also approximates the level of selenium which might be present in swine rations in seleniferous areas where grains contain from 10 to 20 p.p.m. of selenium. Arsanilic acid was used at a level of 0.01% of the total ration for Lot 3.

One boar was used for all three lots. Gilts that did not conceive with the first service were rebred during the next heat period. Those females that showed a third estrus period were bred again and then slaughtered and examined for genital abnormality.

The groups were confined to small lots of approximately ⅓ acre each. A moderate stand of brome grass was available to the pigs during the first 5 to 6 weeks of the experiment. During lactation the sows and litters were confined to dirt dry lots with their first litter and on concrete with their second litter. One gilt from Lot 1 was accidentally killed on the 56th day of the experiment. The growth data from this gilt was

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included for the time she was on the trial. All data were treated statistically by analysis of variance as outlined by Snedecor (1946).

Results and Discussion

Growth Data. During the first 8 weeks of the experiment an apparent response was obtained from the arsanilic acid as the pigs receiving the ration containing selenium and arsanilic acid gained at a faster rate (1.21 lb. per day) than did the control group or the group receiving the ration containing 10 p.p.m. of selenium. Since the latter two groups gained at approximately the same rate, 1.05 and 1.09 lb. per day, it appears that the selenium was not altering the growth rate. Previous work at this Station

TABLE 1. PERCENTAGE COMPOSITION OF RATIONS

Ingredients	Initial wt. to 150 lb.	From 150 to 250 lb.	From 250 lb. through gestation	During lactation
Ground yellow corn	84.0	41.5	21.5	64.5
Ground oats	....	41.5	41.5	12.0
Ground alfalfa hay	....	10.0	30.0	10.0
Soybean oil meal	10.0	4.0	4.0	8.0
Tankage	5.0	2.0	2.0	4.0
Trace mineral salt	0.5	0.5	0.5	0.5
Steamed bonemeal	0.5	0.5	0.5	1.0
B-vitamin supplement[a]	0.1	0.1	0.1	0.1

[a] Provided the following additions to each pound of ration: 1 mg. riboflavin, 2 mg. panthothenic acid, 4.5 mg. niacin, 5 mg. choline and 4.5 mcg. vitamin B_{12}.

(Wahlstrom *et al.*, 1955, 1956) indicated a reduction in rate of gain when 10 p.p.m. of selenium was incorporated in the ration. A possible explanation is that the consumption of brome grass diluted the selenium content of the total feed intake. When the grass was no longer available, the pigs were older and heavier and, therefore, less susceptible to selenium poisoning.

At a weight of 200 lb. the average daily gains were 1.45 lb. for the controls, 1.36 for the selenium group and 1.39 for the selenium plus arsanilic acid group. Two pigs in Lot 2 showed visible selenium toxicity symptoms; one of these had only a slight loss of hair while the other gilt had lost all of her hair and the hoofs on all feet had separated ½ in. to 1 in. from the coronary bands. This gilt continued to gain at a slow rate during this time. There were no signs of selenium toxicity in Lot 3 which received the selenized ration plus arsanilic acid. However, the original growth response due to arsanilic acid was not maintained. The rate of gain from the time of an average weight of 200 lb. until farrowing was similar for all groups.

Breeding and Gestation. The effect of selenium and arsanilic acid on the breeding performance of first litter gilts is presented in table 2. Of the nine gilts in the control lot, eight conceived with the first service and one failed to conceive after three services. Five of the 10 gilts in Lot 2, which received the selenized ration, conceived with the first service, two more

with the second service and three gilts failed to conceive after three services. The ovaries of two of these gilts were cystic when examined at slaughter and the ovaries of the other showed no signs of corpora lutea, although this gilt had been in heat 4 days prior to slaughter. It appeared that the ovaries were non-functional and ovulation had not occurred. The gilts in Lot 3, which received the selenized ration plus arsanilic acid, showed estrus behavior similar to those in Lot 2. Only four of the gilts in Lot 3 conceived at the first service, four conceived after the second service, and one gilt failed to conceive after three services. The ovaries from this gilt also were

TABLE 2. THE EFFECT OF SELENIUM AND ARSANILIC ACID ON BREEDING PERFORMANCE OF SWINE

Comparison	Lot number and treatment		
	1	2	3
	Basal	Basal+ Selenium (10 ppm)	Basal+ Selenium (10 ppm) +Arsanilic Acid (0.01%)
No. gilts	9	10	10
No. conceived	8	7	8
No. services/conception[a]	1.0	1.3	1.5
No. farrowed	7[b]	6[c]	8
No. days from weaning to estrus	8	13	32

[a] Gilts that conceived.
[b] One gilt aborted on the 108th day of gestation.
[c] One gilt aborted on the 54th day of gestation.

examined and appeared to be non-functional. The remaining gilt in this lot died of a urinary infection; she had been bred once, but the autopsy revealed that she had not conceived.

Farrowing and Lactation. Farrowing and lactation performance are summarized in table 3. The control gilts farrowed an average of 8.0 live and no stillborn pigs as compared to 7.7 live and 2.1 stillborn, and 6.6 live and 0.9 stillborn farrowed by those fed the selenium and selenium plus arsanilic acid rations, respectively. The average birth weight as well as vigor of the pigs was reduced when selenium was included in the maternal ration. Arsanilic acid did not appear to be of any benefit in counteracting this effect. There was also a greater death loss between birth and weaning (56 days) in Lots 2 and 3 than in Lot 1. Statistical analysis showed that Lot 1 weaned significantly more ($P < 0.01$) of the total pigs farrowed and also significantly more ($P < 0.05$) of those farrowed alive than did Lots 2 and 3. Average number of pigs lost during the lactation period was 0.7 in Lot 1, 2.0 in Lot 2 and 1.6 in Lot 3. Thus, gilts in Lot 1 weaned an average of 91.2% of the pigs farrowed while gilts in Lots 2 and 3 weaned 57.6 and 66.7% of the total number farrowed, respectively, or 73.9 and 75.5% of the pigs farrowed alive. Decreased survival of the progeny of rats receiv-

ing selenium has been reported by Franke and Potter (1936) and Rosenfeld and Beath (1954).

Westfall *et al.* (1938), using the rat and cat as experimental animals, reported that selenium is transmissible through the placenta to the mammalian fetus. The passage of selenium into the milk has been reported by McConnell (1948). The reduced growth rate of the young pigs in Lots 2 and 3 could well have been due to their receiving selenium in these two ways. It is true that these pigs did consume some of the selenized rations fed to the sows. However, it was observed that the pigs in Lots 2 and 3

TABLE 3. THE EFFECT OF SELENIUM AND ARSANILIC ACID ON REPRODUCTION AND LACTATION OF SWINE

Items	No. litters farrowed	Av. no. pigs farrowed	Av. no. pigs farrowed alive	Av. birth wt., lb.	Av. no. pigs weaned	Av. weaning wt., lb.
Basal						
First litters	7	8.0	8.0	2.95	7.3	30.5
Second litters	6	11.2	10.5	2.89	8.0	29.0
Average	6.5	9.6	9.25	2.92	7.65	29.75
Basal+Selenium						
First litters	6	9.8	7.7	2.60*	5.7	23.1**
Second litters	5	8.6	7.2	2.76	5.6	23.5**
Average	5.5	9.2	7.45	2.68	5.65	23.3
Basal+Selenium+Arsanlic Acid						
First litters	8	7.5	6.6	2.55**	5.0	24.6**
Second litters	6	9.0	8.7	2.76	6.0	27.4
Average	7	8.25	7.65	2.66	5.5	26.0

* Significantly less than basal lot ($P<.05$).
** Significantly less than basal lot ($P<.01$).

were not growing as rapidly as those in Lot 1 before much feed was being consumed by the pigs themselves. The average litter weights at 56 days of age were 222, 131 and 140 lb. for Lots 1, 2 and 3, respectively. This would represent a great economic loss to the swine producer due to selenium toxicity.

Second Litters. After the first litters were weaned the sows were rebred for second litters. A marked difference existed in length of time from weaning to estrus as is shown in table 2. This factor did not appear to have any effect on conception. One sow in each of Lots 1 and 2 did not conceive after two services and two sows in Lot 3 failed to conceive with the first service but conceived when rebred during the second estrus cycle. These sows were removed from the experiment, and account for the lower number of sows farrowing second litters.

The farrowing performance of the sows farrowing their second litters is also shown in table 3. The effects of selenium were again evident in the percentage of dead pigs farrowed and in average birth weight of pigs from the Lot-2 sows. The Lot-3 sows farrowed very few dead pigs but lost a higher percentage of pigs from birth to weaning time. An enteric disorder in one litter of pigs from the Lot-1 sows caused the death of six pigs. This decreased the average number of pigs weaned by Lot-1 sows

from nine to eight. Lot-1 sows fed the basal ration farrowed 94% live pigs and weaned 72% of the total number farrowed or 76% of the number farrowed alive. Lot 2 farrowed 84% live pigs and weaned 65% of the total number farrowed or 78% of those farrowed alive. Lot 3 farrowed 97% live pigs but weaned only 66% of the total number farrowed or 68% of those farrowed alive. These differences in number of pigs weaned were not statistically significant.

The average weaning weights were heavier for pigs from sows fed the basal ration. At 56 days of age these pigs averaged 5.5 lb. heavier than the pigs of the sows in Lot 2 and 1.6 lb. heavier than those of sows in Lot 3. Average litter weights were 232, 132 and 164 lb. for Lots 1, 2 and 3, respectively. This is in close agreement with the average weights of the first litters.

Summary

Thirty weanling Duroc gilts were used to study the effect of selenium on swine reproduction and the value of arsanilic acid as a selenium poisoning preventive. Ten p.p.m. of selenite selenium lowered the conception rate and increased the number of services required per conception. Sows fed this ration farrowed a higher percentage of dead pigs and their pigs were smaller and weaker at birth. Fewer pigs were weaned and the 56-day weights of these pigs were significantly reduced.

Arsanilic acid in the selenium ration resulted in an improvement in percentage of pigs farrowed alive but did not improve the birth weights or average number of pigs weaned per litter. The greatest advantage of arsanilic acid appeared to be in increasing the weight of pigs at 56 days.

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THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF VARIOUS PORK HAM MUSCLE CLASSES [1, 2]

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TWO-TONED color in pork ham muscle has been a controversial issue during recent years. Wilson (1954) conducted myoglobin analyses of ham muscles from hogs of known heritage and nutrition and found two-toning to be related to the breed of hog, but not to the feeding regimen. Extreme variations in pork muscle appearance were also reported by Ludvigsen (1954, 1955). This worker has devoted extensive study to a condition termed "Muscular Degeneration," which he characterized as a sour smell, low pH, watery appearance and pale grayish color. Various transitional two-toned forms ranging from this condition to a dark, firm, dry appearing tissue were also noted. Recently, Self *et al.* (1957) reported that pork ham two-toning was not related to weight, sex, carcass length, percent lean cuts or loin grade.

The purpose of this paper is to present data showing the chemical and physical characteristics of certain pork ham muscle classes ranging from pale, soft watery tissue to dark, firm, dry appearing tissue.

Experimental

The samples for this study were secured from hogs slaughtered in a local processing plant, and from hogs slaughtered at the University Meats Laboratory. The latter group had been subjected to various experimental treatments. The pork carcasses, ranging in weight from 138 to 160 lb., were chilled for a 24-hour period at 34°—38° F. Subsequently, the hams were removed from the carcasses by cutting between the second and third sacral vertebra, perpendicular to the shank. The exposed ham butt muscles were classified 24 hr. after slaughter in accordance with the following muscle descriptions.

I. "Pale-two-toned" muscles were pale in color, soft and watery in structure and possessed loose inter-muscular binding.

II. "Two-toned" muscles adjacent to the bone were dark in color, while the large outer muscles were pale in color, lacking in firmness, moderately soft in structure and moderately watery immediately after cutting.

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[3] Departments of Animal Husbandry and Biochemistry, co-operating.

III. "Normal" muscles were uniform in color (grayish-pink) and moderately firm in structure.

IV. "Dark" muscles were firm in structure and moderately to extremely dark in color. Muscles were dry immediately after cutting.

The *gluteus medius* (light outer ham muscle) and the *gluteus accessorius*, and *gluteus profundus* (dark inner muscles) were selected for detailed analyses.

Physical Determinations. A model "G" Beckman pH meter was used to determine the pH values of the muscle samples at 2 minutes, 40 minutes and 24 hours after slaughter.

Measurements of the expressible water were made by modifying the rapid method proposed by Grau and Hamm (1953). This method is hereby termed the "Filter Paper Moisture Absorption Technique". The modified apparatus (figure 1) consisted of plexiglass plates which were

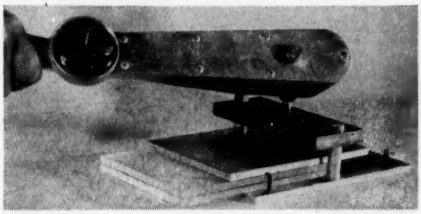

Figure 1. Filter paper moisture absorption apparatus.

placed between two ¼-inch aluminum sheets. A ⅜-inch bolt (16 threads per in.) was centered to afford a constant pressure area. Precautions were taken to reduce errors due to individual variations in the moisture content of the filter paper. When the sample was prepared for measurement, a filter paper sheet was singularly removed from a humidifier and placed between the plexiglass plates. The sample (0.3 gm) was immediately placed on the midportion of the paper. Two-hundred pound inches of torque were applied to the bolt through the use of a torque wrench, thus applying a force of 4,350 lb. to the top plate. The muscle and water areas were subsequently measured with a polar planimeter and the relative amount of expressible water was recorded as the ratio of the muscle area to the water area. (figure 2). This measurement was made on samples 40 minutes and 24 hours after slaughter.

Chemical Determinations. Three-inch portions of the muscles were ground 3 times in an Oster food grinder. The samples were thoroughly mixed between each grinding. The fat and moisture percentages were determined by the modified A.O.A.C. procedure as reported by Kelly (1955). Total myoglobin concentrations were determined by the method of Ginger *et al.* (1954). Quantitative modification of the nephelometric method

of Hansen *et al.* (1952), provided a rapid determination of muscle glycogen. The fresh tissue samples were removed 2 minutes after the animals lost consciousness, and were immediately placed between two angular Dry-ice portions in a plastic insulated container. Subsequently, the frozen samples were analyzed for glycogen. Sodium and potassium were determined by a modified procedure of Toth *et al.* (1953) with the use of model 52 (Perkin-Elmer) Flame Photometer. These determinations were conducted on samples 24 hours after slaughter. The data were analyzed statistically by the analysis of variance method as outlined by Snedecor (1950).

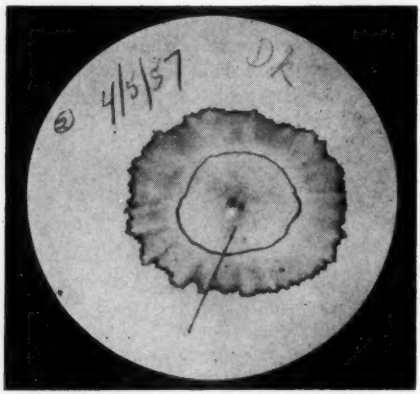

Figure 2. Muscle Area and water area for determining expressible water ratios. a. Muscle area; b. Expressible water area.

Results

Tables 1 and 2 summarize the characteristics of the *gluteus medius* and the *gluteus accessorius* and *gluteus profundus* of the four ham muscle classes.

Although the myoglobin concentrations were slightly higher in class IV, there were no significant increases due to muscle class. This was true for the *gluteus medius,* as well as, the *gluteus accessorius* and *gluteus profundus* muscles.

The pH values of the fresh tissue 2 minutes after slaughter were similar for all muscles regardless of the final classification. The pH values of the *gluteus medius* muscles from hams in classes I and II, 40 minutes after slaughter, were significantly ($P<0.05$) lower than those of muscles from classes III and IV hams. The increase in acidity appeared to continue in classes I and II during the 24-hr. chilling process. At the conclusion of the chilling period (24 hours), the pH values of the *gluteus medius* muscles of classes I and II were significantly ($P<0.01$) lower than those in classes III and IV. A similar situation existed for the *gluteus accessorius* and

TABLE 1. CHEMICAL AND PHYSICAL CHARACTERIZATION OF *GLUTEUS MEDIUS* (LIGHT OUTER) MUSCLE

Chemical and physical factors[a]	Ham muscle classes			
	I (Pale-two-toned)	II (Two-toned)	III (Normal)	IV (Dark)
pH, 2 min. after slaughter	6.19 (6)[d]	6.36 (7)	6.29 (12)	6.35 (7)
pH, 40 min. after slaughter	5.73 (18)	5.90 (11)	6.09 (16)*	6.07 (15)*
pH, 24 hr. after slaughter	5.42 (20)	5.53 (20)	5.61 (20)	5.93 (20)**
Glycogen, mg./gm.				
At 2 min. after slaughter	9.52 (6)	11.77 (7)	5.59 (12)	2.15 (7)**
At 24 hr. after slaughter	2.34 (6)	0.57 (7)	0.89 (12)	0.31 (7)*
Expressible water ratio,[b]				
At 40 min. after slaughter	1.55 (6)	1.46 (7)	1.25 (12)	1.56 (7)
At 24 hr. after slaughter	2.57 (6)	2.69 (7)	2.39 (12)	2.25 (7)
Myoglobin,[c] mg./gm.	0.91 (20)	0.99 (20)	1.11 (20)	1.20 (20)
Sodium,[c] %	0.44 (11)	0.31 (17)*	.28 (10)*	0.34 (32)*
Potassium,[c] %	1.46 (11)	1.25 (17)	1.22 (10)	1.15 (32)

[a] All quantitative chemical data are expressed on a fat-free, dryweight basis.
[b] Water area to muscle area, see figure 2.
[c] At 24 hr. after slaughter.
[d] Number of observations for each factor and class are in parentheses.
* Significantly different, at the 5% level, from the underlined observation (s).
** Significantly different, at the 1% level, from the underlined observation (s).

TABLE 2. CHEMICAL AND PHYSICAL CHARACTERIZATION OF *GLUTEUS ACCESSORIUS* AND *GLUTEUS PROFUNDUS* (DARK INNER) MUSCLES

Chemical and physical factors[a]	Ham muscle classes			
	I (Pale-two-toned)	II (Two-toned)	III (Normal)	IV (Dark)
pH, 2 min. after slaughter	6.20 (6)[d]	6.33 (7)	6.42 (12)	6.26 (7)
pH, 40 min. after slaughter	5.88 (18)	6.04 (11)	6.30 (16)*	6.29 (15)*
pH, 24 hr. after slaughter	5.72 (20)	5.93 (20)	5.95 (20)	6.19 (20)**
Glycogen, mg./gm.				
At 2 min. after slaughter	6.61 (6)	5.55 (7)	4.21 (12)	2.29 (7)
At 24 hr. after slaughter	2.26 (6)	.52 (7)	1.56 (12)	.72 (7)*
Expressible water ratio,[b]				
At 40 min. after slaughter	1.51 (6)	1.51 (7)	1.37 (12)	1.53 (7)
At 24 hr. after slaughter	2.07 (6)	2.08 (7)	1.77 (12)	1.92 (7)
Myoglobin,[c] mg./gm.	3.10 (20)	2.69 (20)	2.84 (20)	3.44 (20)
Sodium,[c] %	.36 (11)	.35 (17)	.34 (10)	.39 (32)
Potassium,[c] %	1.71 (11)	1.25 (17)	1.23 (10)	1.18 (32)

[a] All quantitative chemical data are expressed on a fat-free, dryweight basis.
[b] Water area to muscle area, see figure 2.
[c] At 24 hr. after slaughter.
[d] Number of observations for each factor and class are in parentheses.
* Significantly different, at the 5% level, from the underlined observation (s).
** Significantly different, at the 1% level, from the underlined observation (s).

gluteus profondus; however, the significant difference was only exhibited by the extreme classifications (classes I and IV).

Conversely, the glycogen concentrations of the *gluteus medius* muscles were significantly higher in classes I and II than in classes III and IV, 2 minutes after slaughter. There also seemed to be a trend in this direction in the *gluteus accessorius* and *gluteus profundus* muscles although the differences were not significant.

There were no consistent differences in potassium concentrations in the muscles analyzed. There were, however, significant increases in the sodium concentration of the *gluteus medius* muscles of class IV.

Although, there were no significant differences in the expressible water ratios among the four classes of hams, all four classes showed increases in the ratio of the water areas to the muscle areas during the chilling process.

The expressible water ratios decreased as the pH values approached 7.0 and therefore, were especially low in the *gluteus medius* muscles of class IV. This trend was not paralleled by a similar pattern in the *gluteus accessorius* and *gluteus profundus* muscles.

Discussion

The myoglobin values were within the lower ranges reported by Ginger *et al.* (1954). It should be noted that there were no significant increases due to muscle class. Since the main visual criterion for muscle class distinction was that of color, special attention was directed to the absence of increased pigment concentration. The *gluteus accessorius* and the *gluteus profundus* contained significantly greater ($P<0.01$) quantities of myoglobin than the *gluteus medius* muscles. Lawrie (1950) suggested that muscles with the same myoglobin content can vary in appearance depending on the muscle pH. Muscles, for example, appear much darker at pH 7.0 than at pH 6.3. He concluded that the principal factor contributing to myoglobin increases in muscles was the demand for oxygen as a result of increased activity, either by growth or exercise. This postulation, suggests an explanation for the wide variation in myoglobin concentration within muscle classes and also between individual muscles.

The pH values, glycogen quantities and expressible water ratios will be concurrently discussed in view of their significant relationships. The similarity of pH values of all fresh tissues regardless of the ultimate muscle classification supports the common contention that the pH of live tissue is physiologically controlled within limits compatible with the biological system. McCarthy and Mackintosh (1953) suggested that muscle pH represented a balance between (a) the buffering capacity and (b) the presence of acidic substances. Likewise, Ludvigsen (1955) reported that the total amount of lactic acid formed was of minor importance in producing pale muscles when compared to the ability of the acid to lower the pH value.

The data herein reported are in agreement with the data reported by Ludvigsen (1955) on the total carbohydrate content of the pork musculature. The data in table 2 shows that the *gluteus accessorius*, and *gluteus profundus* were less variable in character than the *gluteus medius* (table 1). Likewise, the initial (2 minutes) glycogen concentration and the 24-hour expressible water ratios were measurably lower in the *gluteus accessorius* and *gluteus profundus* (table 2).

There were no significant differences between classes in muscle expressible water in the fresh tissue stage. These findings seemed to be related to the similarity in pH values at this stage. The relative amount of expressible water recorded as a ratio of the water area to the muscle area, however, increased significantly during the chilling process. This increase was the greatest in muscles which possessed high concentrations of glycogen at the time of slaughter. Apparently, however, the factors which permit or prevent total glycogen conversion during glycolysis were more important in controlling the appearance than the exact total amount of glycogen stored at the time of slaughter. The fact that the chilled glycogen concentrations were extremely variable, and were the highest in muscles from class I, suggests that glycogen retention in the chilled form was of little value to the muscle condition. Enzyme activity and buffer capacity apparently were implicated in acidity development and final ham classification.

In general, muscle class was a reflection of the amount of glycogen converted during glycolysis subsequent to slaughter. The soft, pale, watery muscles possessed high initial glycogen, low ultimate pH, high expressible water, and relatively high sodium concentration. The fact that these characteristics provided soft, watery, pale tissue was in complete agreement with Hamm's (1955) first postulation on muscle color. He suggested that with a low pH the water would be released, the structure would become more dense, and the light rays would be reflected from the surface layers and make the muscle appear light in color. This is assumed to be the type of reaction that was taking place in these muscles. The understanding of the cause and control mechanisms of these muscle classes requires further study.

Summary

Certain chemical and physical characteristics of the *gluteus medius* and *gluteus accessorius* and *gluteus profundus* ham muscles were studied. The samples were secured from hams ranging in quality from a pale, soft, watery condition to a firm, dark, dry appearing tissue. The physical analyses included pH and expressible water, whereas, the chemical aspects included determinations of myoglobin, glycogen, sodium and potassium. Although some of the determinations were made on both the fresh and chilled tissue, the final ham muscle classification was made 24 hours after slaughter.

The fresh tissues were not classified since the appearance differences were not detectable 2 minutes after slaughter. Likewise, there were no differences in muscle pH at slaughter time. Marked differentiation in muscle character appeared only after the reactions of anaerobic glycolysis had produced considerable acidity. The *gluteus medius* muscles of class I (pale-two-toned) possessed high initial glycogen concentrations, low ultimate pH values, high expressible water ratios and relatively high sodium concentrations. Conversely, the *gluteus medius* muscles of class IV (dark) were characterized by low initial glycogen concentrations, high ultimate pH values, and low expressible water ratios. Muscle color variations between the four muscle classes could only in part be associated with total myoglobin concentrations. The *gluteus accessorius* and *gluteus profundus* muscles were less variable in appearance and character than the *gluteus medius* muscles.

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THE EFFECT OF VARIOUS LEVELS OF EXERCISE IN ALTERING THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF CERTAIN PORK HAM MUSCLES [1, 2]

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IT has been reported by Lawrie (1950) that active pigs have darker muscles of greater myoglobin concentration than inactive pigs. Bull *et al.* (1942) showed that light exercise had no effect on beef muscle color. Recently, Howard *et al.* (1956) subjected steers to severe exercise by having them driven by a mounted stockman for one-half to one and one-half hours immediately before slaughter. Although the muscle glycogen was depleted, the ultimate pH was normal.

The most significant difference between soft, watery, pale pork muscle and dark, firm, dry appearing pork muscle is the initial muscle glycogen concentration. (Briskey *et al.*, 1958). Therefore, it seemed that the depletion of muscle glycogen prior to slaughter would serve as a means of producing dark, firm, dry appearing pork muscle. The control animal exerciser as described by Finner *et al.* (1958) was constructed to force exercise and alter the muscle glycogen concentration.

The purpose of this paper is to present data showing the effects of various levels of forced exercise on the appearance and the physical and chemical characteristics of certain pork ham muscles.

Experimental

Experiment I. Various Levels of Forced Exercise. Sixteen Spotted Poland China gilts averaging 190 lb. were randomly allotted into four lots each of four pigs. The animals were fed a 16% protein basal ration for a two-week period. Subsequent to this period the hogs were designated for the following lots: Lot I, control; Lot II, 1 mile per day (4 days); Lot III, 2 to 3 miles per day (4 days); Lot IV, 3 miles, prior to slaughter.

The exercise for Lot II and III was given twice daily for 4 consecutive days with the exerciser designed by Finner *et al.* (1958). Lot IV received no treatment until immediately before slaughter at which time the hogs were exercised for 3 hours at the rate of 1 mile per hour. As the exercise treatment was completed the animals were slaughtered and the carcasses chilled for 24 hours at 36–38° F.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
[2] From a portion of a thesis presented by E. J. Briskey to the Graduate School, University of Wisconsin, in partial fulfillment of the requirements for the Ph.D. degree, 1958.
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Three-inch long rectangular portions of the *gluteus medius* were removed for analysis. The following observations and determinations were made in accordance with the methods referred to by Briskey *et al.* (1958): Muscle class, myoglobin concentration, pH values, sodium, potassium and total moisture percentages. Photographic standards and word descriptions were used to classify the chilled muscles according to the following classification: (Briskey *et al.*, 1958) I, Pale-two-toned; II, Two-toned; III, Normal; IV, Dark. The Hunter color and color difference meter was

TABLE 1. EFFECT OF VARIOUS LEVELS OF FORCED EXERCISE ON HAM MUSCLE CHARECTERISTICS. EXPERIMENT I—CHEMICAL AND PHYSICTAL DATA FOR *GLUTEUS MEDIUS* (LIGHT OUTER) MUSCLE [a]

Lot and treatment	Lot I Control	Lot II 1 mile/day 4 days	Lot III 2–3 miles/day 4 days	Lot IV 3 miles prior to slaughter
Muscle classes [b]	4 (I)	2 (I) 1 (II) 1 (IV)	4 (I)	3 (IV) 1 (III)
L value (Hunter color scale) [c]	36.08	35.06	37.42	32.90*
Myoglobin,[d] mg./gm.	.94	1.01	.81	1.18
pH (40 minutes after slaughter)	5.57	5.37	5.92	6.27*
pH (24 hr. after slaughter)	5.48	5.51	5.55	5.80*
Sodium,[d] %	.317	.312	.375	.452
Potassium,[d] %	1.39	1.33	1.59	1.68
Moisture, %	70.34	69.53	69.71	69.64

[a] Four animals per lot.
[b] Muscle classes (Briskey *et al.*, 1958). I=Pale-two-toned; II=Two-toned; III=Normal; IV=Dark.
[c] Zero represents pure black and 100 represents pure white.
[d] Fat-free, dry tissue.
* Significantly different at the 5% level from the underlined observations.

also used to record the "L" values of the chilled samples. The "L" value represents the color intensity with 0 being pure black and 100 being pure white. The standard 5 R 3/6 was used with a 1/4 in. aperture opening.

Experiment II. Severe Exercise Under Full-Fed and Fasted Conditions. Twelve Poland China barrows ranging from 200 to 250 lb. were randomly allotted into three lots each of four pigs. Subsequently, the allotted hogs were treated as follows: Lot I, control; Lot II, full-fed and exercised 3 miles immediately prior to slaughter; Lot III, fasted 72 hours and exercised 3 miles prior to slaughter.

Immediately after treatment the hogs were slaughtered at the University Meats Laboratory. After the scalding operation and within 40 minutes from the time of bleeding, the fresh *gluteus medius* samples were removed from the right side for pH and glycogen determinations. The carcasses were subsequently chilled 24 hours and the samples were similarly removed for

further analyses. The analyses of the chilled samples included: Muscle class, "L" values, myoglobin, pH, glycogen, and total moisture percentages. These observations and determinations were conducted in accordance with the methods referred to by Briskey *et al.* (1958). All data were analyzed by the analysis of variance method as outlined by Snedecor (1950).

Results

Effect of Various Levels of Forced Exercise. The data in table 1 imply that the hogs in Lots II and III adjusted to the repeated exercise over

TABLE 2. EFFECT OF SEVERE EXERCISE UNDER FASTED AND FULL-FED CONDITIONS ON HAM MUSCLE CHARACTERISTICS. EXPERIMENT II—CHEMICAL AND PHYSICAL DATA FOR *GLUTEUS MEDIUS* (LIGHT OUTER) MUSCLE [a]

Lot and treatment	Lot I Control	Lot II Full feed, exercised 3 miles prior to slaughter	Lot III Fasted 72 hr., exercised 3 miles prior to slaughter
Muscle classes [b]	2 (I) 2 (II)	1 (II) 1 (III) 2 (IV)	2 (III) 2 (IV)
L value (Hunter color scale) [c]	35.47	31.35*	31.00*
Myoglobin,[d] mg./gm.	1.04	1.09	1.17
pH (40 minutes after slaughter)	5.69	6.08*	5.99*
pH (24 hr. after slaughter)	5.58	6.01*	5.85*
Glycogen [d] (40 minutes), mg./gm.	9.71	2.49*	1.82*
Glycogen [d] (24 hr.), mg./gm.	1.42	.133	.250
Moisture, %	70.60	71.23	72.02

[a] Four animals per lot.
[b] Muscle classes (Briskey *et al.*, 1958). I=Pale-two-toned; II=Two-toned; III=Normal; IV=Dark.
[c] Zero represents pure black and 100 represents pure white.
[d] Fat-free, dry tissue.
* Significantly different at the 5% level from the underlined figures.

a four-day period. There was a high degree of similarity in several muscle characteristics of the hogs from Lots I, II and III. On the other hand, these data implied that the Lot IV treatment was an effective means of altering the ham muscle characteristics. The *gluteus medius* muscles of Lot IV possessed a higher pH and a darker color (Hunter color value). Differences in total moisture, myoglobin, sodium, and potassium concentrations were not significant.

Effect of Severe Exercise Under Full-Fed and Fasted Conditions. Table 2 presents a summary of the characteristics of the *gluteus medius* muscle. These data substantiate the previous finding, mainly, that severe exercise increased the intensity of the color and raised the muscle pH. The

myoglobin concentration, however, was not significantly increased in Lot II and III.

Discussion

Lawrie (1950) and Hall (1944) reported that exercise increased myoglobin concentration. This may account for the slightly higher myoglobin values in Lot IV samples (table 1). The fact that the increase in the intensity of the color was significant (Hunter color value and muscle class score) without a significant increase in myoglobin was further explained by Lawrie (1950) on the basis that myoglobin appears darker at a high pH than at a low pH.

The low pH of the muscles from the hogs in Lots II and III (Experiment I) shows that the animals adjusted to the stress and reaccumulated their glycogen stores. This is in agreement with the postulation of McCarthy and Mackintosh (1953) that the immediate effect of exercise on muscle is a decrease in glycogen content. Therefore, less glycogen is present to form acidic substances, which accounts for the high pH in Lot IV. However, it seems that as the animal becomes adapted to the repeated work, and, if nutrients are available, the glycogen is replenished as the work proceeds. Apparently, this type of physiological response has taken place in hogs from Lots II and III of this experiment.

The high pH of samples from the exercised animals was readily explained by the significantly decreased glycogen concentration (Experiment II). These data were not in agreement with the findings of Wilcox *et al.* (1953) that muscle glycogen concentrations were not related to pH values. Conversely, the data agree precisely with the theories of McCarthy and Mackintosh (1953), Lawrie (1950) and Ludvigsen (1955) that glycogen stores are related to muscle pH. Severe exercise was effective in altering the muscle classification of both the full-fed and fasted hogs.

Summary

Market weight hogs from two experiments were given various levels of forced exercise through the use of a control animal exerciser. The effects of this type of stress on the ham muscle classification and certain chemical and physical characteristics of the *gluteus medius* muscle at slaughter and subsequent to a 24 hr. chill were studied. When repeated exercise was given, the hogs seemingly adjusted themselves rapidly to this type of treatment. The hams from these hogs were very similar to the controls in appearance and characteristics. Fasted and full-fed hogs that were subjected to single, severe exercise possessed muscles that were dark in color, high in pH value and dry in appearance. The exercise treatment on both fasted and full-fed animals significantly decreased initial muscle glycogen. As the muscle glycogen was decreased, the pH value was raised and the muscles appeared dark in color.

The increase in the intensity of the muscle color of the severely exercised lots was not solely a reflection of increased pigment quantities. It is postulated, however, that this increase in color intensity was due to a change in muscle consistency as a result of its higher pH.

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BLOOD CHANGES OF CATTLE DURING BLOAT AND EFFECTS OF THE ALFALFA FORAGE *IN VITRO* [1]

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THEORIES have been advanced that bloat in ruminants is caused by toxic substances in legumes (Heath and Park, 1953; Committee on Animal Health, 1956; United States Department of Agriculture, 1957). While much effort has been directed towards the isolation and pharmacologic testing of chemical constituents of legumes, little work has been reported for the effects of legume forages on blood constituents of cattle.

In this study, various blood constituents were determined on cattle in dry lot and after grazing alfalfa or blue grass pastures. Changes in blood constituents were then related to the pasture grazed and to the occurrence of bloat. Samples of the grazed forage were also tested for their effects on *in vitro* biological systems. It was thus possible to relate changes of both *in vivo* and *in vitro* biological systems to the occurrence of pasture bloat.

Experimental

Twelve cattle of mixed breeding, including two pairs of identical twins, were allowed to graze daily throughout a two-month experimental period for 2 hours in the morning and another 2 hours in the afternoon. At other times the animals were placed in dry lot, with water available. This is the management plan developed by Barrentine *et al.* (1956). The cattle were observed for the occurrence and severity of bloat and were graded immediately after grazing. The following bloat scale was used: No bloat, 0; definite rumen distention, 1; marked rumen distention, 2; marked rumen distention with unsteady gait or movement, 3; and unable to stand, 4 (not encountered in these experiments.) Bloat data are presented only for 12 afternoon grazing periods on the alfalfa pasture and 12 afternoon grazing periods on the blue grass pasture when complete blood studies were made and forage samples were collected. The alfalfa studies preceded the blue grass studies; the test days were selected so as to allow time for the laboratory analyses.

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[4] The authors are grateful to Dr. Melvin Carter, AES Statistical Office, for conducting the statistical analyses.

A blood sample was collected from each animal before the morning grazing period and again after the afternoon grazing. The blood was withdrawn into a heparinized syringe to prevent clotting. The following values were determined for each sample: Total plasma cholesterol (Sperry and Webb, 1950), plasma ascorbic acid (Consolazio *et al.*, 1951), and red cell cholinesterase activity (Michel, 1949). The differences between morning and afternoon values were used as criteria of the effect of the forage on these blood constituents.

In a preliminary experiment with six cattle, nine blood constituents (ascorbic acid, total cholesterol, esterified cholesterol, CO_2, Ca, Mg, Na

TABLE 1. BLOOD CONSTITUENTS OF CATTLE SUBJECT TO BLOAT

Pasture	Bloat score	No. of cases	Red cell cholinesterase A.M.	Red cell cholinesterase Change after grazing	Plasma ascorbic acid A.M.	Plasma ascorbic acid Change after grazing	Plasma total cholesterol A.M.	Plasma total cholesterol Change after grazing
			units [a]	units [a]	mg. %	mg. %	mg. %	mg. %
A. Blood constituents as to forage species [b]								
Blue grass	0	144	1.38	0.00	0.71	−0.04	96.1	−2.5
Alfalfa	0–3	144	1.43	−0.02	0.83	−0.04	86.5	+11.5
B. Blood constituents as to each bloat score on alfalfa [c]								
Alfalfa	0	93	1.41	0.00	0.82	−0.02	85.4	−1.2
Alfalfa	1	32	1.49	−0.07	0.85	−0.10	92.3	+15.7
Alfalfa	2	15	1.47	−0.04	0.83	−0.06	78.2	+63.3
Alfalfa	3	4	1.51	−0.04	0.94	−0.08	93.3	+79.0

[a] Michel, 1949. A unit equals the decrease in pH of a standard buffer in two hours due to enzymatic release of acetic acid from acetyl choline.
[b] Change in cholesterol on alfalfa was significantly different than that on the blue grass.
[c] Change in cholesterol was significantly correlated with the bloat score.

and K in plasma, and cholinesterase in red cells) were determined before and after grazing ladino clover. The blood constituents which changed appreciably in relation to bloat were selected for the present study.

Samples of forage, collected after the afternoon grazing period, were fractionated by a solvent system; the aqueous fraction, thus obtained, was assayed for its *in vitro* effect on acetylcholinesterase and on the oxygen uptake of rat diaphragm muscle (Shaw and Jackson, 1957).

In order to compare the alfalfa and blue grass pastures, data were analyzed as a completely randomized experiment. Since the observations were taken on different pastures and at different times, any differences due to the forage species would be confounded with possible variations due to these features. Thus in the analysis the effects were assumed to be non-existent or small compared to the effects due to the forage species themselves. Since the bloat scores are discreet and are probably not normally distributed, correlations of the blood constituents with the bloat scores were done by the use of rank correlations.

Results and Discussion

Blood Changes of Cattle. Data for the blood constituents of the cattle are summarized in table 1 on the basis of the pasture grazed (blue grass or alfalfa) and the grade of bloat observed (alfalfa). Of 51 cases of bloat observed during the afternoon grazing periods on alfalfa, 32 were grade 1, 15 were grade 2, and 4 were grade 3. No bloat was observed on blue grass. When changes in the blood constituents were compared for the two pastures there was no significant difference in the activity of red cell cholinesterase

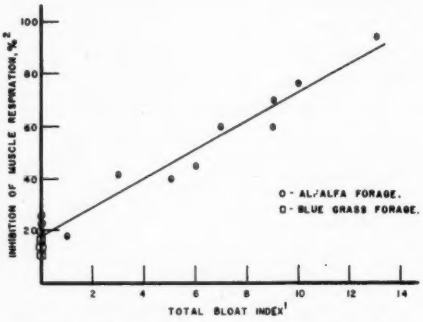

Figure 1. Inhibitory action of forage in vitro vs. the bloat index for that forage.

[1] Algebraic sum of the bloat grades for all 12 animals grazing the forage on the afternoon it was collected.
[2] Based on μl O_2 taken up by 100 mg. rat diaphragm in presence of forage extract as compared to control (Shaw and Jackson, 1957).

(ChE) or content of plasma ascorbic acid. However, total plasma cholesterol content increased 11.5 mg. % on the alfalfa pasture but decreased slightly, 2.5 mg. %, on the blue grass pasture. The changes in blood cholesterol levels in the cattle after grazing the alfalfa were significantly different from the changes observed after grazing the blue grass pasture.

The blood changes of the animals on alfalfa pasture were also compared for each grade of bloat. Ninety-three cases of grade 0, 32 of grade 1, 15 of grade 2, and 4 of grade 3 bloat were observed during the afternoon grazing periods on alfalfa. When bloat was observed red cell ChE activity and plasma ascorbic acid values were slightly depressed but were not correlated with the severity of bloat. However, there was a positive correlation of the total plasma cholesterol values with the bloat scores indicating that the plasma cholesterol increased with increasing severity of bloat.

Increased blood cholesterol levels may possibly be due to the animal's response to a toxic constituent in the alfalfa and in turn the cholesterol

may be related to the animal's detoxification mechanism. Since the decrease in red cell ChE activity showed no correlation with bloat, it appears that anticholinesterase compounds were probably not the causitive agents for the bloat that occurred during these experiments.

Inhibitory Effects of Forage. Data from the *in vitro* assays of the alfalfa forage were compared with the total bloat index of that forage during the afternoon of collection. The total bloat index of a forage is the algebraic sum of the bloat grades for all 12 animals on the afternoon that that forage was grazed. This index varied from day to day. The alfalfa fractions inhibited ChE activity of the test system by as much as 20% but the inhibition was not correlated with the total bloat index. Three composite samples of blue grass (each representing four afternoon collections) inhibited ChE only slightly. These results support the *in vivo* ChE data which suggests that an anticholinesterase compound was not a causitive factor in the bloat observed during these experiments.

The total bloat index of the forage was also compared to the *in vitro* inhibition of respiration of rat diaphragm caused by the forage fractions (figure 1). The inhibition of muscle respiration by the alfalfa fractions was positively correlated with the total bloat index (significant at 1% level, correlation 0.97). These results strongly suggest that the forage components which inhibit muscle respiration *in vitro* are related to bloat. Data (figure 1) also show that the blue grass samples caused only a modest inhibition of the muscle respiration *in vitro* and compare favorably with data from the alfalfa forage on days when no bloat occurred.

A comparison of the bloat index with daily precipitation and temperature during the experimental period did not reveal any consistent correlations between the occurrence of bloat and these weather conditions. The bloat scores (sum of the bloat grades) of each of the animals for the 12 grazing days also differed. The observations indicate that animal susceptibility as well as day to day variations of the forage are important factors in the occurrence of bloat.

Summary

Twelve cattle of mixed breeding were grazed for 12 days on alfalfa pasture and 12 days on blue grass. Blood values were determined for red cell cholinesterase (ChE), plasma ascorbic acid, and total plasma cholesterol before a two-hour morning grazing period and again after a two-hour afternoon grazing period. The animals were graded for bloat after grazing.

Alfalfa pasture increased the total plasma cholesterol values and this increase was highly correlated with the severity of bloat. The occurrence of bloat was highly correlated with the *in vitro* inhibition of muscle respiration produced by a fraction of the forage. No significant change was noted for either ChE (both *in vivo* and *in vitro* systems) or ascorbic acid during bloat.

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THE EFFECT OF EXOGENOUS PROGESTERONE ON FORMATION AND MAINTENANCE OF THE CORPORA LUTEA AND ON EARLY EMBRYO SURVIVAL IN PREGNANT SWINE [1, 2]

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THE importance of progesterone in the establishment and maintenance of early pregnancy is well recognized (Allen and Corner, 1929). It is possible, however, that progesterone may not always be found at the appropriate level or in the proper ratio with other hormone levels for greatest reproductive efficiency. Progesterone therapy of "repeat-breeder" cows has shown a tendency to decrease the large amount of embryonic death by which these cows are characterized (Wiltbank *et al.*, 1956; Herrick, 1953; Dawson, 1954). Pomeroy (1955) suggested from data collected by Perry (1954) that the greater proportion of embryonic death in larger litters of swine than in smaller may be traceable to the larger numbers of corpora lutea and a presumed excess of progesterone. Thus the existence of an optimal progesterone level may be implied.

It has been reported that 30–40% of the ova in swine are lost before parturition and 75% of this loss occurs by the 25th day of gestation (Perry, 1954). Baker *et al.* (1958) estimated survival at the 25th day of gestation was 70%, while by the 70th day it was 52%. When they based survival on pregnant sows only and did not include cases of presumed total embryonic mortality, the percentages were 79% and 62% at 25 and 70 days gestation respectively. Squiers *et al.* (1952) on a similar basis calculated survival at 25 days gestation as 64%, and at term as 54%.

It has been shown that a high TDN intake by gilts from approximately 3 months of age until 25 days of gestation is harmful to embryo survival as compared to a moderate TDN intake (Robertson *et al.*, 1951; Christian and Nofziger, 1952; Self *et al.*, 1955). Haines *et al.* (1957) changed gilts from a full-fed ration to one furnishing 50% less energy at the time of breeding and administered 25 mg. of progesterone every other day from breeding to 25 days gestation. They found no differences in embryo survival due to hormone treatment or ration.

The purpose of experiment I was to determine the effects of exogenous progesterone upon early embryo survival and upon corpus luteum formation and maintenance in early pregnancy in swine. Experiment II was to determine whether the administration of progesterone during early preg-

[1] Paper from the Department of Genetics No. .... and the Department of Animal Husbandry, published with the approval of the Director of the Agriculture Experiment Station.

[2] We would like to acknowledge the advice and aid given by R. G. Loy in the chemical analysis of the corpora lutea.

nancy might diminish the difference in early embryonic death producible by the long-time self feeding of low-fiber and high-fiber rations.

Experiment I

Materials and Methods

Eighty-two market gilts of mixed breeding weighing from 180 to 260 lb. were assigned at random to 12 experimental groups. The gilts were checked

TABLE 1. EXPERIMENTAL DESIGN, AVERAGE INDIVIDUAL CORPUS LUTEUM WEIGHT, PERCENT EMBRYO SURVIVAL, PROGESTERONE CONCENTRATION, PERCENT I-II TYPE CELLS, AND WITHIN CLASS CORRELATIONS OF CELL TYPE AND PROGESTERONE CONCENTRATION

Group	Trial	No. of animals	Days of gestation when— Injected[a]	Slaughtered	A Av. individual corpus luteum wt. (mg.)	B Percent embryo survival[e]	C Progesterone concentration (mcg./gm.)	D Percent I–II type cells	rcd[d]
I	A	5	none	4	small (5)[b]	98 (5)	.....	.....	....
II	A	5	0–4	4	cystic thin-walled[c] (5)	100 (5)	.....	.....	....
III	B, C	8	none	18	402 (8)	72 (8)	24 (4)	45 (4)	0.68
IV	B, C	8	0–18	18	189 (4)	1 (8)	17 (3)	29 (3)	0.49
V	B, C	8	4–18	18	282 (8)	96 (8)	27 (4)	41 (4)	0.95
VI	B, C	8	none	25	413 (8)	83 (8)	33 (4)	80 (4)	0.17
VII	B	4	0–25	25	.....	0 (4)	.....	.....	....
VIII	B, C	8	0–4	25	242 (8)	5 (8)	17 (3)	26 (3)	0.93
IX	B	4	0–18	25	.....	0 (4)	.....	.....	....
X	B, C	8	4–18	25	250 (8)	22 (8)	32 (3)	57 (3)	0.13
XI	B, C	8	4–25	25	233 (7)	72 (8)	29 (2)	21 (2)	1.00
XII	B, C	8	18–25	25	188 (8)	79 (8)	16 (4)	9 (4)	0.90

Within groups=0.83**(P<.01)
Between groups=0.76*(P<.05)

[a] One mg. of progesterone daily per lb. body weight injected s/c dissolved in corn oil at a concentration of 25 mg. per cc.

[b] Number in parenthesis denotes number of animals per group for which data were obtained.

[c] Percent solid corpora lutea differed significantly between groups I and II at the 5% level by the t test.

[d] Correlations calculated using angle of percent I and II type lutein cells.

[e] The percent for each individual gilt was transformed to an angle and the average angle of the group then transformed to percent.

for heat once daily and bred as they exhibited estrus by one of two boars known to be fertile. All the gilts on treatment were weighed at the beginning of injections and received progesterone daily at the rate of 1 mg. per lb. body weight during planned intervals (see table 1 for intervals) between breeding and the 25th day of gestation. The hormone, injected subcutaneously, was dissolved in corn oil at a concentration of 25 mg. per cc. The numbers of animals and treatments for the various groups are shown in table 1. All the gilts received the same ration *ad libitum* throughout the experiment which included three successive trials designated as A, B, and C.

In trial A groups I and II only were studied. They were slaughtered 96 hours after breeding and the ova were recovered and studied under a low power binocular miscroscope. The percentages of eggs that appeared as normally developing embryos and the characteristics of the corpora lutea were recorded.

Trial B consisted of groups III through XII and involved four animals per group. Groups III through V were slaughtered on the 18th day of gestation and groups VI through XII on the 25th day of gestation. Embryo and membrane weights were taken on the 25-day animals post mortem. The excess embryonic fluids were drained on blotter paper before weighing. Corpora lutea were counted, dissected from the ovary, and weighed in those animals in which corpora lutea could be distinguished at the time of slaughter. The corpora lutea had regressed and could not be distinguished from earlier corpora albicantia in groups VII and IX.

Trial C was conducted to study the histological structure (cell types) and progesterone content of the corpora lutea in addition to weight of the corpora lutea and embryo survival. It consisted of four animals per group and was conducted in the same manner as trial B except groups VII and IX were omitted.

All the corpora lutea from each gilt were weighed and the average weight of an individaul corpus luteum calculated. A single corpus luteum was selected at random from each gilt for histological study and the remainder were stored in 95% ethanol at approximately —6° C. for later analysis of progesterone content using the method of Loy *et al.* (1957). Data on three animals (two in group XI and one in group IV) were lost as a result of an accident during chemical analysis and the corpora lutea from two animals (one in each of groups VIII and X) were not analyzed chemically because of insufficient total tissue for the determination. All other group means of progesterone concentration and cell types were based on four animals per group.

The corpus luteum from each gilt used in the histological study was fixed in Bouin's solution, sectioned and stained with haemotoxylin and eosin. The lutein cells were classified into five different types using the classification of Foley and Greenstein (1958) for the young corpus luteum of the bovine. Types I and II cells with vesicular nuclei were combined as were also types III, IV, and V with pyknotic and shrunken nuclei. A differential count of five microscopic fields (magnified 1000 X) for each corpus luteum and the percent of type I plus type II out of the five types of lutein cells was calculated. These percentages, transformed to angles, were used in the statistical analysis.

Embryo and membrane weights were compared in groups VI, XI, and XII. Embryo and membrane weights from other 25-day groups were not compared because of the high mortality rate. Average individual corpus luteum weights, embryo survival, and embryo and membrane weights from comparable groups of trials B and C were pooled for the statistical analysis.

Results and Discussion

No significant effects on fertilization rate due to progesterone injections were evident (table 1). The percent of ova recovered in the two groups and their location in the uterine horn (measured in 6-inch segments of the horn starting at the tubo-uterine junction) did not differ significantly. In the 4-day control group 71% of the ova were recovered, 97% of these in the first 6 inches of the uterine horn; in the treated group 82% of the ova were recovered, 91% of these in the first 6 inches of the uterine horn. The remainder of the ova in these two groups (3% and 9% respectively) were recovered in the second 6 inches of the uterine horn. Pomeroy (1955) suggested that excess progesterone may increase the rate of travel of the ova through the Fallopian tube in the pig, but the present observation does not support the suggestion.

TABLE 2. MULTIPLE RANGE TEST FOR EMBRYO SURVIVAL TRANSFORMED TO ANGLES (5% LEVEL) [a]

Group	IV	VIII	X	III	XI	XII	VI	V
Mean	4.0	12.9	28.2	57.8	57.9	62.9	65.4	78.0
N	8	8	8	8	8	8	8	8

[a] The test at the 1% level showed no differences between groups IV, VIII, and X or between X, III, and XI, but was otherwise the same.

MULTIPLE RANGE TEST FOR CORPORA LUTEA WEIGHT (5% LEVEL) [a]

Group	XII	IV	XI	VIII	X	V	III	VI
Mean	188	189	233	242	250	282	402	413
N	8	4	7	8	8	8	8	8

[a] The test at the 1% level was the same except that groups V and III did not differ significantly.

A significantly larger proportion of solid corpora lutea was observed in the control group than in the progesterone treated group (table 1). The percentages were 65 and 21, respectively. A large proportion of the corpora lutea in the progesterone treated group was pulpy or fluid-filled with only slight luteinization of the walls. This abnormal formation of corpora lutea may have been due to the inhibition of luteinizing hormone. Foote *et al.* (1958) found evidence that release of the luteinizing hormone was inhibited in gilts injected with progesterone.

Progesterone administered at various stages from day of breeding to the 25th day of gestation showed no significant beneficial effect on embryo survival when compared with controls at 18 or 25 days gestation (table 2). Groups VII and IX in which embryo survival was nil and the corpora lutea

had regressed were not included in the formal statistical analysis, but in a comparison of the other groups a lower survival was found in groups IV, VIII, and X (and presumably in groups VII and IX) when compared with the remaining groups ($P<.01$). Groups IV and VIII had a significantly lower survival than group X ($P<.05$). Sammelwitz *et al.* (1956) also observed poor survival at 25 days pregnancy in gilts injected with approximately the same level and twice the level of progesterone as used in this study.

Progesterone injections started on the day of mating were detrimental in every case, except group II (groups IV, VII, VIII, IX). It appears that exogenous hormone had produced some harmful effects on the embryo or its environment by the fourth day but these effects were not visible in the animals of group II which were slaughtered at 96 hours of gestation. Possible related observations in the literature are Whitten's (1957) finding of toxic effects of progesterone on embryonic development *in vitro* and Cochrane and Meyer's (1957) observation that progesterone without estrogen in the ovariectomized rat maintained embryonic life but did not bring about implantation. Neither of these factors can be evaluated in this study.

In group X survival may have been lowered due to withdrawal of exogenous progesterone after having brought about regression or lowered function of the corpora lutea. The progesterone concentration and percent of I and II type cells in the small corpora lutea present in the gilts of this group still pregnant at 25 days did not differ significantly from the controls although the percent I and II type luteal cells was somewhat lower.

The progesterone treatments decreased the average individual corpus luteum weight significantly in every case as compared to the two control groups (table 2). The 18- and 25-day control groups did not differ greatly (table 1, 402 mg. vs. 413 mg.). The progesterone treatment did not produce significant differences in the means of the groups in progesterone concentration or percent I and II type cells, but there was a significant difference in the amount of variance within the treated groups as compared to the two control groups in percent I and II type cells ($P<.05$). This difference was not significant with regard to progesterone concentration.

The correlation of percent I and II type cells and progesterone concentration was calculated for each group (table 1). The sums of squares and sums of cross products were then pooled and the resulting within group correlation was 0.83 (table 1, $P<.01$). The between group correlation was of a similar magnitude, 0.76 (table 1, $P<.05$).

The lowered individual corpus luteum weight, the increase in variance of cell type within treated groups as compared to controls, and the high association between cell type and progesterone concentration (of similar magnitude between and within groups) would appear to indicate that exogenous progesterone had caused regression in size and lowered function of the corpora lutea.

TABLE 3. AVERAGES OF EMBRYO WEIGHTS AND MEMBRANE WEIGHTS

Group	VI	XI	XII
Embryo weight,[a] gm.	0.42	0.48	0.43
Membrane weight,[a] gm.	6.6	8.5	8.5

[a] Differences were not significant.

Embryo and membrane weights were not significantly different in the three 25-day groups compared (table 3). Foote *et al.* (1957) observed significantly heavier embryos at 25 days gestation in ovariectomized progesterone-estrone treated ewes than in intact ewes at the same stage.

Experiment II

Materials and Methods

Forty-eight gilts 90-days old from the University of Wisconsin herd (24 Chester White and 24 Poland China) were divided randomly into two groups within breed; one group was given a low-fiber ration *ad libitum* and the other a high-fiber ration, *ad libitum* (table 4). The gilts on high-fiber were started on a 24.5% corn cob ration in an attempt to have them gaining 70% as rapidly as the low-fiber lot. They were transferred to a 27.5% cob ration at about 120 days of age when it became evident that they were exceeding the 70% rate of gain. They were changed to a ration containing 40% cobs at an average age of 154 days and kept on that ration throughout the rest of the trial. The crude protein percentage was made equal in the high- and low-fiber rations and was reduced from 16 to 14% when the 40% cob ration was started. The crude protein in the

TABLE 4. RATIONS USED IN THE CHESTER WHITE AND POLAND CHINA FEEDING STUDY IN WHICH HORMONE WAS SUPPLEMENTED

	High-fiber ration			Low-fiber ration	
Ration components	16% protein		14% protein	16% protein	14% protein
	(a)	(b)			
Ground corn	250	220	115	535	585
Ground oats	250	250	250	250	250
Soybean meal (44%)	100	100	90	80	55
Meat scraps (50%)	100	100	90	90	55
Alfalfa leaf meal (17%)	50	50	50	50	50
Ground corn cobs	245	275	400	...	...
Trace mineralized salt	5.0	5.0	5.0	5.0	5.0
Antibiotic mixture	1.0	1.0	1.0	1.0	1.0
Zinc sulfate hepta hydrate	0.5	0.5	0.5	0.5	0.5

[a] 24.5% corn cob ration.
[b] 27.5% corn cob ration.

cobs was disregarded. The high-fiber group averaged 135.5 lb. and the low-fiber group averaged 162.7 lb. at that time.

Twelve gilts on high-fiber and twelve gilts on low-fiber (six of each breed) were assigned randomly to one of two hormone treatments. One group received 1 mg. of progesterone daily per lb. of body weight and the other group 1 mg. daily per 2.5 lb. body weight. The series of injections was started on day-10 of gestation and continued to day-25 at which time the gilts were slaughtered.

All gilts were checked once daily for heat by a vasectomized boar starting at 140 days of age and bred at their second estrous period. The boars used (4 Chester White and 4 Poland China) were mated to the gilts at random with the following restriction, Chester White boars to Poland China gilts and Poland China boars to Chester White gilts.

Injections were given and embryo, membrane, and corpus luteum weights were taken as described for experiment I. Percents of survival, transformed to angles, and embryo, membrane, and corpus luteum weights were analyzed by analysis of variance. The same error term, calculated from all 48 animals, was used in all three parts of the analysis (all animals; within controls; within progesterone treated animals—table 6). The data from the 24 control gilts were also used in another experiment and were therefore dealt with in this study only to see if high-fiber feeding produced a significant advantage in embryo survival over low-fiber feeding and to test if progesterone treatment reduced this mortality in the low-fiber group (treatment-feed interaction). The effect of breed was not considered pertinent in this study. The effect of dosage was studied using only the 24 progesterone treated animals.

Results and Discussion

Percent survival was significantly higher in the high-fiber controls than in the low-fiber controls (tables 5 and 6; means of 71.5 and 54.5, respectively). The treatment-feed interaction was not significant, thus progesterone did not appear to reduce the higher mortality in the low-fiber group (table 6). There was no significant difference between dosage levels in embryo survival within treatment group, nor were the various interactions significant.

Corpus luteum weight was significantly decreased by progesterone treatment (tables 5 and 6). This is consistent with the findings in experiment I. There was no significant effect due to dosage level in corpus luteum weight. This would appear to indicate both dosages were above the normal level found in the body.

The dosage level of progesterone analyzed within the treated animals had no significant effect on embryo or membrane weights (tables 5 and 6). The dosage-breed-feed interactions for embryo or membrane weight were not significant (table 6).

TABLE 5. AVERAGES OF EMBRYO SURVIVAL AND CORPUS LUTEUM, EMBRYO AND MEMBRANE WEIGHTS

Breed	Progesterone[b]	Percent embryo survival[a]		Av. individual corpus luteum weights (mg.)		Membrane weights (gm.)		Embryo weights (gm.)	
		H.F.[c]	L.F.[c]	H.F.	L.F.	H.F.	L.F.	H.F.	L.F.
Chester White	0[d]	86	64[f]	354	392[g]	10.2	10.7[g]	.55	.55[g]
	1.0[e]	81	69	319	265	10.1	7.7	.46	.45
	2.5[e]	52	87	247	310	9.3	9.9	.55	.62
Poland China	0[d]	94	69	428	410	7.7	6.3	.51	.50
	1.0[e]	76	36	255	343[h]	8.0	10.1[h]	.48	.62½
	2.5[e]	75	70	342	333	10.1	7.2	.51	.53

[a] See footnote e table 1.
[b] O=no treatment, 1.0=1 mg. progesterone daily s/c per lb. body wt., 2.5=1 mg. progesterone daily s/c per 2.5 body weight. (In table 6, 0=untreated and 1.0 and 2.5=treated.)
[c] H.F.=High-fiber ration, L.F.=Low-fiber ration.
[d] Averages based on six animals per group unless otherwise indicated.
[e] Averages based on three animals per group unless otherwise indicated.
[f] Based on five animals, one failed to reach puberty.
[g] Based on four animals, one failed to reach puberty and one returned to heat prior to the 25th day of gestation.
[h] Based on two animals, the third gilt had no surviving embryos at 25 days gestation.

TABLE 6. ANALYSIS OF THE EFFECT OF PROGESTERONE AND FEED LEVEL ON EMBRYO SURVIVAL, AND CORPUS LUTEUM, EMBRYO AND MEMBRANE WEIGHT

Sources of variance	d.f.	Mean square			
		Corpus luteum weight	Embryo survival	Embryo weight	Membrane weight
Factorial-all animals					
Treatment	1	106502**	544	0	1.39
Feed	1	2992	1225	0.01	3.45
T×F	1	450	564	0.02	0.27
Factorial-within controls					
Breed	1	.....	182	....	....
Feed	1	.....	1726*	....	....
B×F	1	.....	28	....	....
Factorial-within progesterone treated animals					
Dosage	1	901	83	0.01	0.12
Breed	1	6567	179	0	0.87
Feed	1	2882	63	0.02	2.81
D×B	1	4082	222	0.04	1.75
D×F	1	165	993	0.01	1.36
B×F	1	1820	676	0.01	0.33
D×B×F	1	17014	41	0	24.59
Error	33	5510	398[a]	0.014	9.74

**=P<.01; *=P<.05.
[a]=35 degrees of freedom.

Summary

The effect of exogenous progesterone on early reproductive performance was studied in 130 gilts. The dosages of hormone were 1 mg. per lb. of body weight during various intervals between zero and 25 days of gestation and 0.4 mg. per lb. of body weight daily from day-10 to day-25 of gestation.

Progesterone given daily from time of mating to 96 hours of gestation did not have a significant effect on fertilization rate (97.8% in the controls vs. 100% in the treated), but a higher percent of solid corpora lutea was found in the control group than in the treated group (65 vs. 21; $P<.05$).

Progesterone injected daily during various intervals between zero and 25 days produced no significant beneficial effects on embryo survival. The 18- and 25-day control groups had a significantly higher embryo survival (72% and 83%, respectively) than the two groups treated continuously from breeding to slaughter at the 18th and at the 25th day (1 and 0%), the group treated from breeding to the 18th day and killed on day-25 (0%), the group treated from breeding to day-4 and killed on day-25 (5%), and the group treated from day-4 to day-18 and killed on day-25 (22%). Treatment initiated at day-4 and continued until slaughter on the 18th day as well as treatments initiated on either day-4 or day-18 and continued until slaughter at the 25th day did not appear to have a deleterious effect (means of 96%, 72% and 79%, respectively).

Progesterone treatment decreased the average individual corpus luteum weight significantly in every case as compared to the 18- and 25-day control groups. The correlation of percent luteal cells with vesicular nuclei and progesterone concentration was 0.83 ($P<.01$) between individuals within groups and 0.76 ($P<.05$) between groups.

Embryo and membrane weight did not differ significantly due to progesterone treatment, nor did dosage level have a significant effect upon them.

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THE EFFECT OF EXHAUSTIVE EXERCISE AND HIGH SUCROSE REGIMEN ON CERTAIN CHEMICAL AND PHYSICAL PORK HAM MUSCLE CHARACTERISTICS [1]

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IT has been demonstrated that the ham muscles from hogs exercised severely immediately prior to slaughter were dark in color, firm in structure and dry in appearance (Briskey *et al.*, 1958). This increase in the intensity of the color was not accompanied by significant increases in the myoglobin concentration. The dark color, however, was related to the initial muscle glycogen concentration, and explained in terms of theories presented by Hall (1944), Hamm (1955) and Ludvigsen (1955), who postulated muscle consistency and pH as active mechanisms in the control of muscle color or appearance. The purpose of the experiment reported in this paper was to alter the muscle glycogen concentration and subsequently muscle appearance and character through the use of exhaustive exercise and high sucrose regimens.

Experimental

Thirty-two Poland China barrows ranging in weight from 180 lb. to 240 lb. were secured from commercial sources, fed a 16% protein basal ration for a two-week period, stratified according to weight group, and then randomly allotted into four lots each of eight pigs. The following experimental design governed this experiment: Lot I, control (basal—16% protein ration); Lot II, basal ration and exercised to exhaustion prior to slaughter; Lot III, 50% sucrose ration for 7 days prior to slaughter; Lot IV, 50% sucrose ration for 7 days and exercised to exhaustion prior to slaughter.

Lots II and IV were exercised (Finner *et al.*, 1958) at the rate of 1 mile per hour to exhaustion prior to slaughter. Individual hogs in Lots III and IV were removed 7 days prior to slaughter and given access to a high carbohydrate sucrose ration. The sucrose (50 lb.) was added as a substitute for 50 lb. of corn per 100 lb. of the basal ration. A slaughter order was followed to insure the sampling of one pig per lot per slaughter day.

As the animals were bled, samples of the *gluteus medius, gluteus accessorius* and *gluteus profundus* were removed from the right ham.

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Within 2 minutes from the time of bleeding, pH values were determined and the samples were placed in dry ice for freezing and subsequent glycogen analysis.

The chilled hams were classified according to the muscle classification system discussed by Briskey *et al.* (1958). The chilled sample portions of the *gluteus medius, gluteus accessorius,* and *gluteus profundus* muscles were removed from the left side of the carcasses. The "L" value, myoglobin, pH, glycogen and expressible water were determined on the fresh and

TABLE 1. EXHAUSTIVE EXERCISE UNDER NORMAL AND HIGH SUCROSE REGIMENS.[a] PIGMENT AND CLASSIFICATION DATA. *GLUTEUS MEDIUS* (LIGHT OUTER) MUSCLE

Lot and treatment	Lot I Basal	Lot II Exercise-Basal	Lot III Sucrose	Lot IV Exercise-Sucrose
Muscle classes (chilled samples)[b]	1 (I) 2 (II) 4 (III) 1 (IV)	8 (IV)	4 (I) 3 (II) 1 (III)	1 (I) 2 (II) 4 (III) 1 (IV)
Muscle class average[b]	2.62	4.00**	1.62*[f]	2.62
Cured classification[c]	2.00	4.00**	1.75*[f]	2.50
Myoglobin (fresh)[d]	1.19	1.05	1.11	1.09
Myoglobin (chilled)	1.14	1.00	1.08	1.03
L value (fresh)[e]	30.87	31.75	31.07	33.81
L value (chilled)	39.75*	37.85	40.18*	39.45*

[a] Eight hogs per lot.
[b] Subjective muscle classification using photographic standards and word descriptions; Class I= Pale two-toned; Class II=two-toned; Class III=normal; Class IV=dark. For more detailed descriptions refer to Briskey *et al.*, (1958).
[c] Subjective scale of 1 being light and 4 being dark.
[d] Myoglobin expressed as milligram per gram on a fat-free dry weight basis.
[e] "L" value represents absolute value on the Hunter color scale. Zero represents pure black and 100 pure white.
[f] Lot III significantly different at the 5% level from Lot I and Lot IV.
** Significantly different at the 1% level from the underlined observation (s).
* Significantly different at the 5% level from the underlined observation (s).

chilled tissue by the modified methods previously described (Briskey *et al.*, 1958).

Hams from four animals in each lot were cured and smoked. Color intensity scores were determined on the interior portions of the hams.

Results and Discussion

Chemical analyses data of the *gluteus accessorius* and *gluteus profundus* showed these muscles to be similar regardless of animal treatment. The results and discussion in this paper will be confined to the *gluteus medius* muscles where treatment did affect their chemical and physical characteristics. Pigment and classification data collected on the *gluteus medius* muscles are presented in table 1. The muscle classification data imply that

exhaustive exercise, with normal rations, created dark, dry tissue. In contrast, the high sucrose ration seemed to produce muscles that were pale in color, soft in structure and extremely watery in appearance. When the Lot IV animals were exercised to exhaustion, the muscles were improved to the point where they very closely resembled the muscles of the basal lot. These subjective classification data were supported by the Hunter color values.

TABLE 2. EXHAUSTIVE EXERCISE UNDER NORMAL AND HIGH SUCROSE REGIMENS. CHEMICAL AND PHYSICAL DATA.[a] *GLUTEUS MEDIUS* (LIGHT OUTER) MUSCLE

Lot and treatment	Lot I Basal	Lot II Exercise-Basal	Lot III Sucrose	Lot IV Exercise-Sucrose
pH (2 minutes)[b]	6.34	6.36	6.19	6.32
pH (40 minutes)	6.00	6.16	5.83*	6.12
pH (24 hours)	5.50	5.89*	5.32*[f]	5.48
Glycogen[c] (2 minutes)	8.46	1.97*	10.99	6.17
Glycogen (24 hours)	.547	.350	2.31	.798
Expressible water ratio[d] (Ratio water area to muscle area) (2 minutes)	1.46	1.52	1.46	1.31
Expressible water ratio (Ratio water area to muscle area) (24 hours)	2.66	2.22	2.68	2.28
Moisture, %[e] (24 hours)	73.19	72.92	73.15	73.97

[a] Eight hogs per lot.
[b] Inidicates sample age or the period of time between bleeding and the analysis of the samples.
[c] Milligrams per gram of fat-free dry tissue.
[d] Expressible water ratio as described by Briskey *et al.* (1958).
[e] Total moisture % determined by modified A.O.A.C. procedures.
[f] Significantly different at the 1% level from Lot II.
* Significantly different at the 5% level from the underlined observation (s).

Since the color changes seemed to occur during the chilling period, the myoglobin determinations were conducted at both stages to clarify the position of myoglobin in the creation of muscle color. These data clearly demonstrate that even though there were changes in color, there were no evidences of myoglobin destruction during the chilling process. It is recognized that although the pigment quantity was not increased, the oxidized state of the iron may have been altered.

The cured muscle classification data show that fresh muscle classes distinctively dictate the appearance and structure of the cured product. This is in agreement with data presented by Wilson (1954).

The pH, glycogen and expressible water values are presented in table 2. Although all the muscle pH values were similar at the time of slaughter, the pH of muscles from hogs in Lot III were significantly decreased ($P<0.05$) after 40 minutes had elapsed. At the 24-hour stage these dif-

ferences were highly significant ($P<0.01$). These results support the theories of Hall *et al.* (1944) Lawrie (1950) and Hamm (1955), who suggest muscle consistency and pH as important determinants in muscle color formation.

Whereas, the exercise treatment has been related to muscle color it can also be aligned with glycogen storage (table 2). The pH changes can be explained, in part, therefore, by the differences in the initial glycogen concentration. The basal, unexercised (Lot I) and sucrose-fed, exercised (Lot IV) hogs possessed *gluteus medius* muscles which were very similar in initial glycogen, pH and muscle class. By contrast, the hogs from Lots II and III were significantly different in these respects. Although the glycogen concentration of Lot III was not significantly greater than Lot I and Lot IV, the differences suggest a definite trend toward increased glycogen accumulation upon feeding sucrose. The lower pH values of the muscles from hogs in Lot III can be explained on the basis of initial glycogen concentration.

Work of Howard and Lawrie (1957) showed animals to differ widely in the amount of glycogen conversion during fasting. The data reported herein are in contrast with the work reported by Wilcox *et al.* (1953), who studied the effect of sucrose feeding on the dressing percentage and quality of beef and pork. Although the glycogen concentration was measurably increased, they observed no differences in muscle quality.

Although the expressible water data (table 2) reflect wide variations between samples, major increases appeared during the chilling process. The increase in the expressible water ratio was related to the amount of initial glycogen, and the ultimate pH. The ratio of expressible water increased approximately 0.7 during chilling in Lot II while increases of 1.22 were observed in Lot III. This pH and expressible water relationship supports Hamm's (1953) theory for water binding, namely, that as the pH is lowered, the isoelectric point is approached and the water binding capacity is decreased. Further studies must be conducted to determine the nature of glycogen deposition, glycogen retention and water binding capacities of pork muscle.

Summary

Market weight Poland China barrows were placed on basal and high sucrose regimens, and were given exhaustive exercise immediately prior to slaughter. The effects of these rations and exercise on the characteristics and classications of the *gluteus medius* muscles were studied. Exhaustive exercise of the hogs on the basal ration produced dark, firm muscles that were dry in appearance. These muscles presented high pH values, low glycogen concentrations and relatively low expressible water ratios. In contrast to this response, hogs which were placed on high sucrose rations and given no exercise had muscles that were pale in color, soft in structure and extremely "watery" in appearance. These

muscles were relatively low in pH, high in expressible water and contained approximately five times as much initial glycogen as the Lot II samples. When similarly treated animals were exercised to exhaustion (Lot IV) they possessed hams which very closely resembled the muscles of the basal, unexercised lot. When the hams were cured, the appearance differences were of the same magnitude as in the uncured form.

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ESTROGEN EXCRETION BY COWS AFTER ORAL ADMINISTRATION OF DIETHYLSTILBESTROL[1]

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IN the field of animal reproduction investigations dealing with the metabolism and excretion of estrogens are desirable in elucidating the action of these compounds during the estrous cycle, pregnancy, lactation and anestrum. Furthermore, such studies are particularly important in farm animals because of the use of synthetic substances as components of rations and the presence of active compounds in plant materials used as feed. Burroughs *et al.* (1954) have demonstrated an increase in rate of gain and efficiency of feed utilization in beef cattle fed trace quantities of diethylstilbestrol. Browning *et al.* (1956) reported an increase in milk production by feeding this compound to identical twins and Turner *et al.* (1957) observed an increase in persistency in diethylstilbestrol fed animals in which lactation had been induced by hormonal treatment. However, Wrenn and Sykes (1957) reported that feeding 11 cows 10 to 15 mg. of diethylstilbestrol per day for a period of 60 days had no detectable effect on milk production.

Hisaw and Meyer (1929), Nibler and Turner (1929) and Turner *et al.* (1930) have reported a relatively high estrogenic activity in the urine from pregnant cows as evaluated by animal assays. Smith *et al.* (1956) developed a fluorimetric procedure for estrogen estimation and when applied to the urine of pregnant cows, the total fluorescence produced by this method increased with advancing stages of pregnancy. However, bioassay data on a limited number of samples indicated that about one-fourth of the fluorescence was due to biologically active estrogens.

The study presented here deals with fecal and urinary excretion of estrogen by ovariectomized cows treated with diethylstilbestrol (DES). Data were also obtained on the estrogenic activity of milk from pregnant and non-pregnant cows before and following DES treatment. Assays were also made on urine from a pregnant cow and on bile from non-pregnant cows. The evaluation of estrogenic activity was made by using the response of the uterus of immature mice fed a ration containing the various materials to be tested.

Experimental Procedure

Cows used in this investigation with weights and ages were as follows: Ayrshire (3867) 1020 lb., 3 yr.; Holstein (3991) 970 lb., 3 yr.; Holstein

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(3955) 900 lb., 3 yr.; Holstein (3707) 1300 lb., 5 yr.; Holstein (3970) 1200 lb., 4 yr.; Brown Swiss (3501) 1300 lb., 6 yr. and Guernsey (3804) 1000 lb., 4 yr.

Estrogen excretion studies were conducted on three ovariectomized cows, Ayrshire (A-3867) and Holsteins (H-3991 and H-3955). One intact animal (BS-3501) was also used in this phase of the investigation. These animals were fed a ration consisting of medium quality alfalfa hay with corn, oats, wheat bran and soybean oil meal. A metabolism stall as described by Hansard (1951) was used during 3-day periods of urine and feces collection. Aliquot samples of urine were stored at —14° C. Samples of feces were dried at 90° C. and ground prior to storage. Excreta were collected from animals on the ration and subsequently on the same ration plus DES. The experimental animals received 10 mg. daily for 8 consecutive days. Capsules containing the DES were administered with a balling gun. On the sixth day of treatment the cows were placed in the metabolism stall and collections were made for the following 3 days. At a later time, the cows received 100 mg. daily for 8 consecutive days and excreta collections were made over a 3-day period.

Assays for estrogenic activity were made on milk obtained from cows at different stages of the reproductive cycle. Preliminary comparative assays indicated approximately the same estrogenic activity in milk dried by an infra-red lamp, electric oven, freeze drying or the roll process. Pooled samples of morning and night milkings were dried by the roll process.[3] Samples were collected from H-3707 (3 weeks post-partum) and H-3970 and BS-3501 (both 7 months pregnant). DES was given subsequently to H-3970 and BS-3501 at the rate of 10 mg. per day for 8 days. Milk samples were obtained from each cow on the eighth day of treatment.

One experiment was carried out to observe the effect of feeding DES on the estrogenic activity of bile of the cow. G-3804 (non-pregnant) was given 1 mg. DES daily for 7 days. Gall-bladder bile was collected at slaughter on the seventh day of treatment. Bile was also obtained from a non-pregnant cow not receiving DES.

The estrogenic activity of samples of dried milk, cow ration, feces, urine and bile was estimated by using the uterine weight response of immature mice. The assay procedure was similar to that described by Cheng *et al.* (1953). Materials to be assayed were added directly to a basal ration consisting of 74% yellow corn meal, 20% dried non-fat milk solids, 4% corn oil (Mazola) and 2% mineral mixture (Hubbell, Mendel and Wakeman). Mice weighing 8–10 gm. of the Webster-Swiss strain were used. Treatments were randomly assigned to groups of 8 to 10 mice. The aliquot of urine to be used in each assay level was mixed with approximately 50 ml. of a 4:1 mixture of ethanol and diethyl ether. This solution was

[3] The milk was dried through the assistance of Dr. R. J. Remaley, American Dry Milk Institute, Inc., Chicago; Dr. S. T. Coulter and Dr. C. M. Stine, Dept. of Dairy Husbandry, University of Minnesota, St. Paul.

mixed with 200 gm. of assay ration for each dosage level. Other aliquots of urine were treated with 200 units of beta-glucuronidase per ml. in order to hydrolyze conjugated glucuronides of estrogen metabolites. An incubation period of 24 hours at 37° C. was used. Mouse ration was mixed with weighed amounts of dried milk and feces. An assay of the ration fed the cows was made by feeding it to test animals. Animals were fed *ad libitum* for 6 days and sacrificed on the seventh day. A standard curve was established for each assay. The uteri were removed from the animals, dis-

TABLE 1. ESTROGENIC ACTIVITY OF FECES AND URINE FROM OVARIECTOMIZED COWS FED DIETHYLSTILBESTROL (DES)

Cow	Dosage DES per day, mg.	Feces per day, dry, kg.	Urine per day, liters	Relative potency, mcg.		95% confidence limits	Excreted estrogen as DES per day, mg.	Percent excreted as DES
				per gm.	per ml.			
A–3867	10	2.21		2.72		1.53–3.67	6.01	60.1
			9.24		0.24	0.13–0.30	2.22	22.2
					0.26[a]	0.19–0.30	2.40	24.0
H–3991	10	2.48		1.17		0.83–1.21	2.90	29.0
			8.21		0.25	0.24–0.27	2.05	20.5
					0.37[a]	0.27–0.52	3.04	30.4
H–3955	10	2.26		1.66		1.31–2.09	3.75	37.5
			7.07		0.22	0.17–0.30	1.56	15.6
					0.25[a]	0.19–0.35	1.77	17.7
A–3867	100	3.09		12.84		10.04–15.16	39.68	39.7
			8.30		2.56	2.50–3.00	21.25	21.3
					2.28[a]	1.19–5.48	18.92	18.9
H–3991	100	2.62		9.11		5.27–15.73	23.87	23.9
			7.66		2.46	2.12–2.95	18.84	18.8
					2.39[a]	2.38–2.40	18.31	18.3
H–3955	100	2.25		25.82		15.44–43.17	58.10	58.1
			7.00		1.49	0.70–3.11	10.43	10.4
					2.57[a]	1.90–3.76	17.99	18.0

[a] Sample treated with 200 units of beta-glucuronidase per ml. of urine. Incubation period 24 hours at 37° C.

sected free of adhering tissues, blotted lightly on filter paper, and weighed on a Roller-Smith Torsion Balance. The range in amounts of DES used for each standard curve was 0.40 to 1.02 mcg. per 100 gm. of ration. In a typical recovery assay using DES the relative potency was 1.03 and the confidence limits were 0.85 – 1.19.

Results

Data obtained on the excretion of estrogenic activity by ovariectomized cows are presented in table 1. Results from assays of the ration showed that little, if any, detectable estrogenic activity was present in the feed consumed by each cow. Furthermore, assays of excreta indicated the absence of estrogen in the urine and feces from ovarectomized cows consuming the ration. The results obtained from the assay of milk for estrogenic activity are summarized in table 2. Assay of urine from a cow (BS-3501) in the seventh month of pregnancy indicated a relative potency of 0.0605 mcg. per ml. The average volume of urine excreted per day was

10.21 liters and the total estrogenic activity, calculated as DES, excreted during 24 hours was 0.6177 mg.

Data were obtained on the estrogenic activity of bile. A non-pregnant cow (G-3804), in diestrum, was fed 1 mg. DES daily for 7 days. This animal was slaughtered in the seventh day of treatment and 200 ml. of gall bladder bile collected. This bile was added to the assay ration at the rate of 4.0 and 6.4 ml. per 200 gm. of assay ration. The average uterine weights were 9.5 and 10.7 mg., respectively. Bile was also assayed from a

TABLE 2. ASSAY OF MILK FOR ESTROGENIC ACTIVITY

Assay no.	Cow no.	Stage of reproductive cycle	DES administered daily for 8 days, mg.	Gm. dried milk for 200 gm. assay ration	Av.[a] uterine wt., mg.
1	....	.....	0	0	6.8
2	H-3707	3 weeks post-partum	0	108	6.6
3	"	" " " "	0	192	6.9
4	H-3970	7 months pregnant	0	62	8.6
5	"	" " "	0	100	13.3
6	"	" " "	10	100	10.8
7	"	" " "	10	160	11.3
8	BS-3501	" " "	0	120	8.5
9	"	" " "	0	200[b]	11.8
10	"	" " "	10	100	14.8
11	"	" " "	10	160	17.6

[a] Eight animals.
[b] Assay ration consisted of dried milk.

non-pregnant cow not receiving DES. The secretion was included in the assay ration by adding 10 and 16 ml. to 200 gm. of ration. The average uterine weights were 9.1 and 9.7 mg. respectively. The average uterine weight of the control mice receiving the mouse ration was 6.8 mg. According to Levin (1945) cows, during the last 2 weeks of pregnancy, excrete from 5,000 to 10,000 rat units of estrogenic activity per kg. of dry feces. Calculated as α estradiol this amounts to 0.9 to 1.4 mg. of estradiol per kg. of fecal solids.

Preliminary assays established linearity between log-dose and uterine response. Dosages of both standard and test preparations which would give responses in this range were selected for the assays. The hypothesis of parallelism for the lines corresponding to the standard and test preparations was supported by the data obtained. A linear dosage-response relationship was shown to exist with significant regression coefficients. Doses of both standard and test preparations were used at geometrically progressing levels and consequently the dosages (log-doses) were in arithmetic progression. This facilitated the use of the formula $M=\overline{X}_s-\overline{X}_t-\frac{\overline{Y}_s-\overline{Y}_t}{b}$

in calculating M, which is log R, where R is an estimate of the true relative potency. $\overline{X}_s$ and $\overline{X}_t$ are the mean dosages for standard and test preparations, respectively. The mean responses are designated by $\overline{Y}_s$ and $\overline{Y}_t$ and the estimated regression coefficients by b.

An analysis of variance is presented in table 3 on data in table 1 dealing with the estrogenic activity of feces from cow H-3991 on the 10 mg. level of DES. This analysis is presented as an example of the statistical procedure used in testing the validity of the mouse assay.

TABLE 3. ANALYSIS OF VARIANCE OF ASSAY DATA ON THE ESTROGENIC ACTIVITY OF FECES FROM COW H-3991 ON 10 MG. DES

Source of variation	Degrees of freedom	Mean square	F
Total	47		
Doses	5	4222.434	14.003
Preparations	1	79.053	.262 N.S.
Regression	1	20070.000	66.330
Parellelism	1	92.140	.305 N.S.
Curvature	1	144.550	.479 N.S.
Difference of curvature	1	545.306	1.808 N.S.
Error (within doses)	42	301.522	

N.S.—Not significant

The F values of parallelism, curvature, and difference of curvature are all below the 5% critical values; consequently, the assay may be considered valid as regards linearity and parallelism. Orthogonal contrasts were made when there were equal numbers of mice at each dosage level. Confidence limits of R were determined according to Burn *et al.* (1952). The g-criterion was applied to determine which formula to use in calculating confidence limits. When the g value is less then 0.1 the following confidence limits may be used,

$$M \pm t\sqrt{V(M)}, \text{ where}$$

$$V(M) = \frac{s^2}{b^2}\left[\frac{1}{N_s} + \frac{1}{N_t} + \frac{(M - \overline{X}_s + \overline{X}_t)^2}{S_{xx}}\right]$$

and M has been defined above. When the value of g is greater than 0.1 the following formula for the confidence limits may be used, which is a slight modification of, but equivalent to, that given by Burn *et al.* (1952).

$$M + \frac{g}{1-g}(M - \overline{X}_s + \overline{X}_t) \pm \frac{st}{b(1-g)}\sqrt{\left(\frac{1}{N_s} + \frac{1}{N_t}\right)(1-g) + \frac{M - \overline{X}_s + \overline{X}_t}{S_{xx}}}$$

Designations used are as follows: N_s=number of animals receiving the standard treatment; N_t=number of animals on the test treatment; s^2 is an estimate of the variance obtained from the error mean square in the

analysis of variance, 301.522 in the example given in table 3; b is the regression coefficient, and S_{xx} is the sum deviations of x values from their mean. The g value is calculated from the formula:

$$g=\frac{s^2 t^2}{b^2 S_{xx}}$$

using the same number of degrees of freedom for t as for the mean square error, 42 in the example given in table 3. The method of statistical analysis developed here is similar to that described by Burn *et al.* (1952).

Discussion

The data in table 1 indicate variation in fecal excretion of estrogenic activity by ovariectomized cows fed 10 mg. DES daily for a period of 8 days. For example A-3867 excreted 60.1% of the estrogenic activity consumed whereas H-3991 and H-3955 excreted 29.0% and 37.5%. The same cows excreted 39.7%, 23.9%, and 58.1% of the estrogen intake, respectively, when fed 100 mg. daily for 8 days. On the 10 mg. level of DES administration the combined daily fecal and urinary excretion of estrogen per 100 lb. body weight for A-3867, H-3991 and H-3955 was 0.82, 0.61 and 0.61 mg., respectively, and at the 100 mg. level it was 5.75, 4.35 and 8.45 mg., respectively. These data are not in agreement with the findings of Turner (1956) who reported that dairy cows fed diethylstilbestrol at a 10 mg. level showed as much estrogenic activity in the dried feces as was present in the feed. However, Turner (1956) based his conclusion on a comparison of mean uterine weights and not on relative potency estimates. Assays of urine following beta-glucuronidase hydrolysis showed that more estrogenic activity was conjugated in some cases than in others. For example, in the case of H-3991, after feeding of 10 mg. of DES daily, there was approximately a 30% increase in activity whereas in the same cow fed 100 mg. daily there was little, if any, difference between the enzyme treated and untreated samples. Urinary excretion of estrogenic activity was less than fecal excretion with the exception of H-3991. In this case the activity of the enzyme treated urine was equal to that of the feces. The combined daily fecal and urinary excretion ranged from 55.2% (H-3955) to 84.1% (A-3867) in cows receiving 10 mg. per day of DES, and in the animals fed 100 mg. daily it ranged from 42.2% (H-3991) to 76.1% (H-3955). As shown in table 1 the relative potency of the feces from cows A-3867, H-3991 and H-3955 was increased 4.4, 4.7 and 15.5 fold and the urine 8.8, 6.5 and 10.3 fold, respectively, when the level of DES was increased 10 times.

It is of interest that the ovariectomized cows (A-3867, H-3991 and H-3955) did not excrete estrogen in the feces and urine when not given an exogenous source of activity. According to Gorski *et al.* (1957) extracts of urine samples from an ovariectomized cow showed a significant but

small estrogen response using the Astwood (1938) six-hour assay. However, the extracts were low in activity and the assays showed significant departure from parallelism. Asdell *et al.* (1945) were unable to detect activity in the urine of heifers at estrus, but Turner *et al.* (1930) reported an average of 57 R.U. of estrogen in the urine of cows prior to conception.

The data in table 2 indicate little, if any, estrogenic activity present in the milk from H-3707, 3 weeks post-partum. Assays demonstrated a small amount of estrogen in the milk from two cows in the seventh month of pregnancy (H-3970 and BS-3501). A small increase was found in the average uterine weight of assay animals receiving milk from BS-3501 following the daily oral administration of 10 mg. of DES for a period of 8 days. However, the dose-response relationship was not sufficient to permit a relative potency estimate. The average uterine weights from mice used in the assays of milk from cows receiving 10 mg. of DES per day are in close agreement with assay data reported by Turner (1957). The oral administration of higher levels of estrogenic materials to lactating cows may influence the activity of the milk as indicated by the findings of Voelker and Becker (1957). Their data indicated significant amounts of estrogenic activity in milk from cows implanted with relatively high levels of diethylstilbesterol.

In the case of cow (BS-3501) 7 months pregnant, the total daily urinary excretion of estrogenic activity, calculated as DES, was 0.62 mg. This is lower than the average of 8.6 mg. estradiol equivalent for a 24-hour period reported by El-Attar and Turner (1957) for cows pregnant 185 to 234 days. These investigators used a spectrofluorometric method and the biological activity of the substances they evaluated was not determined.

The excretion of diethylstilbestrol has been studied by several investigators. Wilder and Williams (1948) recovered 35% of a single oral dose in the urine of a rabbit. Jellinck (1954) recovered 8 to 20% of a single injected dose in the urine and 50% in the feces of a rabbit. Hanahan *et al.* (1951) found 77 to 98% of the radioactivity of a single injected dose of labeled compound in the intestinal tract of rats 6 hours after injection. Story *et al.* (1957) studied the estrogen excretion by lambs fed DES. The results of this investigation indicated that 80% of the activity was recovered in the urine and feces. These investigators assumed the biological activity of the excreted estrogen was the same as the compound fed. The chemical nature of the estrogenic activity present in urine and feces of cows fed DES is unknown. Some of the compound may be excreted unaltered. It is also possible that the end products of metabolism may be characterized by more or less estrogenic activity than the DES administered to the cows. The data in table 1 indicate that the daily average percent of the DES intake recovered in the feces and urine was 63% if the results from the two levels (10 and 100 mg.) of estrogen administration are taken into consideration. The fate of the remaining 37% is obscure. According to

Preston *et al.* (1956) there is little accumulation of estrogenic activity in organs of animals fed DES. A most likely explanation would be the destruction of the compound by rumen action and metabolic changes subsequent to absorption.

Summary

Estrogen excretion by the cow has been investigated by using the response of the uterus of the immature mouse to estrogenic activity of feces, urine, milk and bile.

No detectable estrogen was found in feces and urine from three ovariectomized cows. The combined daily fecal and urinary excretion ranged from 55.2 to 84.1% by ovariectomized cows receiving orally 10 mg. of DES per day. The excretion range was from 42.2 to 76.1% when the daily DES intake level was 100 mg. The average percentage of the estrogen intake recovered in the urine and feces per day was 63%, calculated as DES. Treatment of cow urine with beta-glucuronidase increased the estrogenic activity in some cases.

Little, if any, estrogenic activity was found in milk from a cow 3 weeks post-partum. Milk from pregnant cows showed a small amount of activity. A slight increase in the estrogen content of milk was found after DES administration to pregnant cows.

The total daily urinary excretion of estrogen by a cow, 7 months pregnant, was 0.62 mg., calculated as DES. Assays indicated a small amount of activity in gall-bladder bile following the daily administration of 1 mg. of DES for 7 days.

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THE INFLUENCE OF ALUMINUM AND ZINC UPON THE ABSORPTION AND RETENTION OF CALCIUM AND PHOSPHORUS IN LAMBS [1]

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PREVIOUS reports from this laboratory involving radiochemical procedures for the measurement of endogenous fecal calcium (Hansard *et al.*, 1951b, 1954, 1956) and California reports for measurements of endogenous fecal phosphorus (Kleiber *et al.*, 1951; Lofgreen and Kleiber, 1953) have confirmed the validity of these procedures for determining quantitative mineral absorption in farm animals. In conjunction with conventional balance and blood studies, they facilitate investigations of the many dietary factors known to affect calcium and phosphorus behavior in the animal body (Comar *et al.*, 1951; Hansard *et al.*, 1954, 1956).

The possible interference of aluminum in the metabolism of calcium and phosphorus is of interest in view of the suggested use of this element as an aleviator for fluorine poisoning in ruminants. Hobbs *et al.* (1954) have fed 0.5% aluminum sulphate in rations of cattle and sheep for this purpose. Evidence has been presented by several investigators, however, that aluminum as the sulphate depresses the availability of phosphorus to the rat (Jones, 1938; Street, 1942), the guinea pig and rabbit (Cox *et al.*, 1931) and chicks (Deobald and Elvehjem, 1935). McCollum *et al.* (1928) and MacKenzie (1931) have suggested that little dietary aluminum appears to be absorbed and that the deleterious effect which this element exerts is probably confined to the alimentary tract. Increased dietary aluminum has been reported to lower the blood phosphorus levels in guinea pigs, rabbits and chicks (Cox *et al.*, 1931). There is no direct evidence of a similar occurrence in ruminant animals, nor are there data available to indicate what effects, if any, aluminum may have upon calcium assimilation.

Reports on the possible effects of excess amounts of zinc in rations are fewer in number than those concerned with aluminum, although the literature contains occasional reference to suspected cases of zinc toxicity. Using zinc-65, Feaster *et al.* (1954) and Sheline *et al.* (1943) showed that only small amounts of the element were absorbed from the alimentary

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tract of cattle and rats. However, Sadasivan (1952) found that rats fed high levels of dietary zinc showed decreased phosphorus assimilation. The findings of Tucker and Salmon (1955) and Hoekstra *et al.* (1956) have greatly increased the interest in this element and their studies have led to the suggestion that the two elements, calcium and zinc, exhibit antagonism.

This study was concerned with the effects of dietary aluminum and zinc upon the physiological availability of calcium and phosphorus in rations for lambs. Chemical and radiochemical blood and balance data indicative of effects upon endogenous fecal excretions and net absorption of dietary calcium and phosphorus are also presented.

Experimental

Twelve selected wether lambs averaging 37 kg. in weight were placed in metabolism units described by Hansard (1951) for individual 7-day balance studies in four feeding trials. During a 14-day conditioning period all animals were fed 2 lb. of the basal ration, consisting of 33.3% cottonseed hulls, 49.4% corn meal, 10.0% soybean oil meal, 6.6% alfalfa meal, 0.6% iodized salt and 0.1% of a vitamin A and D_2 supplement containing 20,000 I.U. and 2,500 U.S.P. units per gram, respectively. The ration was designed to supply maintenance levels of calcium and phosphorus. However, as consumed, the ration contained 0.18% and 0.21% of calcium and phosphorus, respectively. The calcium content was therefore slightly lower and the phosphorus content slightly higher than the amounts recommended for maintenance by the National Research Council (1957).

At the end of the conditioning period all lambs were weighed, randomly divided into three groups each of four animals and allocated to the three treatments for Trial 1, namely, basal ration, basal ration plus 1.0% aluminum sulphate, and basal ration plus 1.0% zinc sulphate. The animals were maintained on these rations for a 7-day preliminary feeding period, followed by the 7-day collection period. Trial II was a replicate of Trial I, and Trials III and IV differed only in that the rations contained 0.5% of the zinc and aluminum salts, respectively. The animals were rested for 10 days between trials, all animals being maintained on the basal ration prior to being re-randomized for the subsequent trial.

The administration of the calcium-45 and phosphorus-32 was made on the first day of the balance study and marked its commencement. In Trial I, two of the four animals in each experimental group were given a single dose of 500 microcuries of calcium-45 with a specific activity of 15 mc per gram of calcium and a similar amount of high specific activity phosphorus-32 quantitatively administered intravenously into the jugular vein. The remaining six animals received the same quantities of the two isotopes administered orally by means of a stomach tube as described in detail by Hansard *et al.* (1951a). In subsequent trials levels of radioactivity were increased to one millicurie per lamb.

Each animal received 1.0 lb. of its respective feed at 8:00 a.m. and 3:00 p.m. each day, and any food residues were weighed daily prior to the 8:00 a.m. feeding for consumption data. Detailed records of feed intakes, food residues and fecal and urinary outputs were maintained. Feces and urine collections were made routinely each day, the time being to correspond with the initial time of dosing with radioactivity. Blood samples were taken from all animals approximately 40 minutes after dosing intravenously and at 3 hours after dosing orally. Only three of the intravenously and three of the orally dosed animals were sampled on each of the three successive days of each trial. However, blood samples were taken from all animals on the last three days of the 7-day balance period to provide blood equilibrium data for use in calculating the endogenous fecal loss of calcium and phosphorus by the isotope dilution procedure.

The calcium content of all samples was determined by a modification of the procedure of Clark and Collip (1925). Total phosphorus was determined by the method of Fiske and Subbarow (1925). Radiocalcium and radiophosphorus were measured in the sample aliquots by conventional procedures as described by Comar *et al.* (1951). Endogenous fecal calcium and phosphorus excretions were calculated by both the isotope dilution and the comparative balance procedures by the methods described by Hansard (1956).

Results and Discussion

Effects of Zinc and Aluminum upon Calcium Absorption and Retention. The summarized data presented in table 1 show a marked decrease in the net retention of calcium by animals receiving supplementary zinc, as compared with the control animals. This lowered net retention was highly significant ($P<0.01$) at both levels of zinc intake. The effects of aluminum were less clearly defined. At the higher level of intake aluminum caused no significant difference in the net retention of calcium. At the lower level, however, there was a significant ($P<0.05$) decrease in calcium retention.

The apparent digestibilities of the calcium in the ration under the three treatments were consistent with the net retention data. The marked decrease in the apparent digestibility of the calcium in the zinc ration was highly significant ($P<0.01$) at both levels of zinc administration. The decrease occasioned by the aluminum, however, failed to be significant.

The disappearance of the intravenously administered calcium-45 from the blood followed a characteristic curve similar to those presented by Hansard *et al.* (1954) for cattle and showed the establishment of an equilibrium between blood, bone, and gastro-intestinal calcium within 40 hours. Approximately 100 hours after administration of the intravenous dose, the rate of blood disappearance was sufficiently constant to allow the specific activity and the corresponding data for the feces to be used in calculating the endogenous fecal calcium excretion. A high degree of reproducibility marked the blood calcium-45 disappearance data for all

TABLE 1. CALCIUM BALANCE SUMMARY FOR LAMBS RECEIVING DIETARY ALUMINUM AND ZINC

Items	Mean data for Trials I and II			Mean data for Trials III and IV		
	Basal ration	Aluminum ration (1.0%)	Zinc (1.0%) ration	Basal ration	Aluminum ration (0.5%)	Zinc (0.5%) ration
Mean weight of animals, kg.	35.6	33.4	33.7	39.4	39.5	40.7
Chemical balance data, g/day						
Intake of calcium	1.56	1.50	1.14	1.57	1.53	1.50
Fecal calcium	2.09	1.87	2.72	1.67	1.89	2.29
Urinary calcium	0.09	0.27	0.08	0.03	0.10	0.12
Net retention of calcium	−0.63±0.14[a]	−0.65±0.14	−1.66±0.20[b]	−0.13±0.09	−0.45±0.14[c]	−0.90±0.17[b]
Radiochemical data:						
Percent Ca-45 in feces:						
Intravenously administered	28.3	30.2	38.3	27.8	34.7	29.4
Orally administered	77.0	83.8	82.9	65.4	70.3	77.6
Fecal endogenous loss[d]	32.9±2.9	27.7±3.2	45.9±5.2[b]	23.3±3.0	28.6±2.3	33.8±3.7[b]
Inorganic calcium in plasma, %	0.110	0.110	0.107	0.100	0.098	0.095
True digestibility, %[e]	41.9±7.3	43.4±6.4	21.2±4.0[c]	49.8±5.1	48.3±5.1	35.1±4.8[c]

[a] Mean±standard error.
[b] $P<0.01$.
[c] $P<0.05$.
[d] Expressed as mg./kg. body-weight/day.
[e] Calculated as $100-\frac{(\text{Fecal mineral}-\text{Endogenous mineral})\ 100}{\text{Mineral intake}}$.

intravenously dosed animals, irrespective of treatment. Little disturbance of the blood:bone:tissue equilibrium could therefore be attributed to the zinc or the aluminum treatments. The appearance and subsequent disappearance curves for calcium-45 in the blood of orally dosed animals showed greater variability than those for the intravenously dosed animals. This is understandable in view of the complicating factor of gastro-intestinal transfer of calcium-45 to the blood with concomitant transfer of the element to the bones and soft tissues. There was evidence of a lowered initial absorption of calcium-45 in those animals receiving supplementary zinc, compared with those on the basal ration. A less marked decrease in initial absorption was noted for the aluminum-fed animals, along with a more rapid disappearance from the blood.

Figure 1 shows the cumulative levels of fecal calcium-45 throughout the test period for both orally and intravenously dosed animals. The steep initial slope represents primarily unabsorbed calcium, and after 70 hours the loss is largely endogenous in origin. It may be seen that some 65% of the orally administered calcium-45 appeared in the feces during the first 72 hours, a total of 75% being excreted after 7 days. This total cumulative percentage is compounded from the non-absorbed calcium-45 together with a smaller amount which may have been absorbed and re-excreted via the intestinal tract. The results of these studies show a definite reduction in the absorption of calcium-45 in those animals which received dietary zinc. The curve for the aluminum-fed animals indicated a similarly reduced absorption of calcium-45, but the change in slope towards the end of the absorption phase suggests a slight reduction in the re-excreted isotope.

By combining the chemical and radiochemical data, fecal endogenous calcium excretion was calculated by the comparative balance procedure, and the isotope dilution method described by Hansard *et al.* (1957). The fecal endogenous calcium data, expressed as mg. per kg. bodyweight per day are shown in table 1. An analysis of variance (Snedecor 1956) of the combined data shows no significant difference between the two procedures employed in calculating the losses from body sources; the mean endogenous calcium losses being 31.7 ± 1.5 and 32.2 ± 1.5 mg. per kg. per day for the isotope dilution and comparative balance methods, respectively. A significantly higher ($P < 0.01$) level of excretion of endogenous fecal calcium was noted in those animals receiving zinc as compared with the controls, but not when supplementary aluminum was fed. The mean endogenous losses for the four trials were 28.1 ± 1.8, 28.2 ± 1.8 and 39.9 ± 1.8 mg. per kg. per day on the basal, the aluminum, and the zinc diets, respectively. There was a significantly higher ($P < 0.05$) endogenous fecal calcium excretion noted in Trials III and IV as compared with I and II but it is doubtful whether this difference can be attributed to the different levels of feeding of the zinc and aluminum. This contention is supported by the fact that the excretion of animals on the basal ration also varied between the

two sets of trials. It is probable that this is a reflection on the variation in climatic and environmental conditions during Trials I and II as compared with III and IV. The true digestibility of the dietary calcium

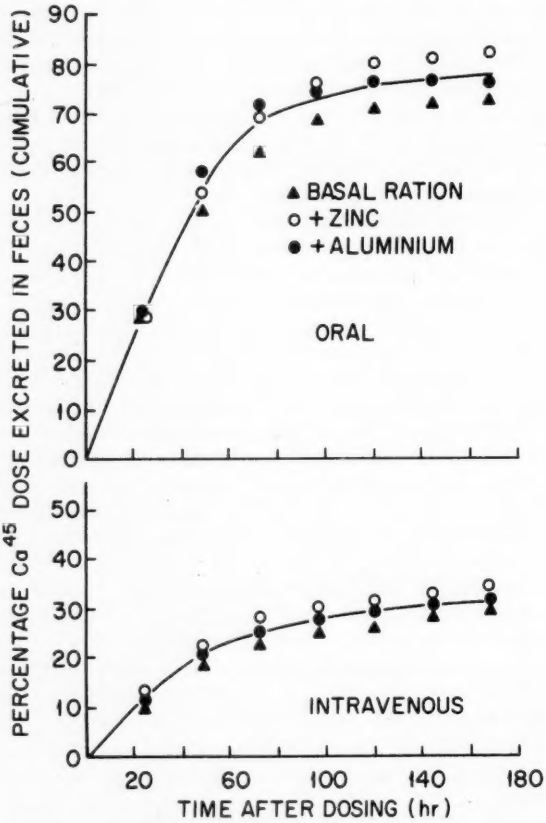

Figure 1. Cumulative fecal excretion of orally and intravenously administered calcium-45 in lambs on basal, high zinc and high aluminum rations.

appeared to be little affected by the administration of aluminum, however, zinc caused a highly significant depression ($P<0.01$) in absorption.

An inspection of the above data shows that the high fecal excretion of calcium in animals receiving zinc can be accounted for, in part, by the increased fecal endogenous calcium loss by animals on this treatment.

TABLE 2. PHOSPHORUS BALANCE SUMMARY FOR LAMBS RECEIVING DIETARY ALUMINUM AND ZINC

Items	Mean data for Trials I and II			Mean data for Trials III and IV		
	Basal ration	Aluminum ration (1.0%)	Zinc (1.0%) ration	Basal ration	Aluminum ration (0.5%)	Zinc (0.5%) ration
Mean weight of animals, kg.	35.6	33.4	33.7	39.4	39.5	40.7
Chemical balance data, g/day						
Intake of phosphorus	2.01	1.95	1.49	1.76	1.72	1.69
Fecal phosphorus	2.06	2.07	1.92	1.64	1.80	2.09
Urinary phosphorus	0.05	0.04	0.07	0.04	0.03	0.05
Net retention of phosphorus	−0.10±0.17[a]	−0.17±0.09	−0.50±0.07[b]	+0.08±0.04	−0.11±0.08	−0.44±0.06[b]
Radiochemical data:						
Percent P-32 in feces:						
Intravenously administered	28.5	27.0	24.2	26.1	28.7	31.3
Orally administered	39.5	44.7	39.5	31.8	37.2	39.0
Fecal endogenous loss[d]	52.5±5.0	48.3±2.3	47.4±4.5	33.8±1.3	38.8±2.7	41.1±2.1[c]
Inorganic phosphorus in plasma, %	0.088	0.081	0.092	0.076	0.076	0.071
True digestibility[e]	88.3±3.0	76.6±4.9	81.6±2.1	84.9±3.4	79.8±2.8	75.3±4.6

[a] Mean±standard error.
[b] $P<0.01$.
[c] $P<0.05$.
[d] Expressed as mg./kg. body-weight/day.
[e] Calculated as $100-\frac{(\text{Fecal mineral}-\text{Endogenous mineral})\ 100}{\text{Mineral intake}}$.

Even so, the decreased true digestibility suggests a further effect of the zinc in lowering the actual absorption from the gastro-intestinal tract. This would appear to support the view that the elements, calcium and zinc, are to some extent antagonistic. As a result, high concentrations of zinc have apparently prevented the normal rate of calcium absorption, possibly by preferential absorption. That the two elements do exhibit some degree of antagonism is supported by the findings of Hoekstra *et al.* (1956) where diets high in calcium prevented the utilization of zinc by swine.

The Effects of Zinc and Aluminum upon Phosphorus Absorption and Retention. Data for the phosphorus absorption and excretion in lambs, as influenced by the feeding of aluminum and zinc, are summarized in table 2. The results of the chemical balance showed a lowering of the net retention at both the low and high levels of dietary zinc when compared with their respective control. However, it must be noted that the incorporation of zinc into the ration particularly at the high level, tended to reduce the appetite and consequently the phosphorus intake. No doubt some of the apparently lowered net retention is attributable to this cause.

The apparent digestibility of phosphorus in the different rations were consistent with the net absorption data. The decrease in the apparent digestibility caused by the zinc feeding is highly significant ($P<0.01$) at both levels of administration. The disappearance of intravenously administered phosphorus-32 from the blood followed the same characteristic curve as that for calcium-45. The phosphorus nuclide appeared to be removed from the blood at a slightly less rapid rate than was the calcium-45, an observation supported by the findings of Shroder (1957) for lambs of similar ages.

Figure 2 shows the cumulative phosphorus-32 contents of the feces from orally and intravenously dosed animals. It may be seen that only some 40% of the orally administered phosphorus-32 appeared in the feces. The intravenous and ingested phosphorus-32 was excreted at a slightly slower rate by the zinc- and aluminum-fed animals than by the control animals. The differences were small, however, and the final cumulative percentages of phosphorus-32 were the same on all treatments.

Despite the apparent differences in the calculated endogenous fecal phosphorus excretions as calculated by the isotope dilution and comparative balance procedures, these differences failed to attain significance. The pooled data obtained gives an endogenous fecal phosphorus output of 40.7 ± 2.0 and 45.6 ± 2.0 mg. per kg. bodyweight for the isotope dilution and comparative procedure, respectively. Treatment appeared to have no significant effect upon the endogenous fecal outputs, which were 42.1 ± 2.4, 43.6 ± 2.4 and 44.2 ± 2.4 mg. per kg. per day, for the basal, the aluminum and the zinc rations, respectively, for the combined trials.

No significance could be attached to differences in true digestibility of the phosphorus in the three rations, but the trend was toward lowered true digestibilities in those rations containing supplemental zinc and aluminum, at both levels of administration.

The overall pattern of phosphorus absorption and retention appears to have been little affected by the inclusion of even 1% of zinc and aluminum sulphates. Certainly in the case of the latter compound only minor effects may be noted; the net retentions, endogenous fecal phosphorus excretions and true digestibilities differ little from those of animals on the basal

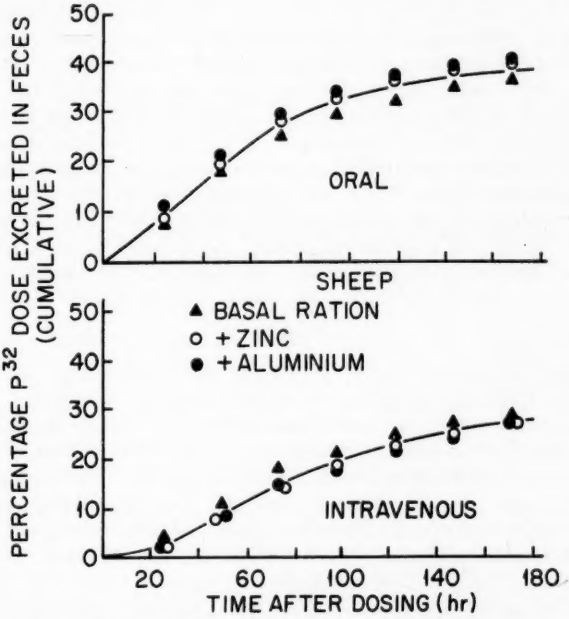

Figure 2. Cumulative fecal excretion of orally an intravenously administered phosphorus-32 in lambs on a basal, high zinc and high aluminum rations.

ration. Zinc does appear to have affected the net retention of phosphorus, but to have caused little change in the losses from body sources. The differences in net retention along with the dependent apparent digestibilities must, to some extent, be questionable in view of some of these differences which could be accounted for by the lower intake of phosphorus on this ration. However, it appears certain that if the lowered net retention data truly reflect the effect of zinc, then it is due to lowered absorption rather than to increased excretion of endogenous phosphorus.

The finding that aluminum caused little or no effect upon the true digestibility of the phosphorus in lamb rations was surprising and at variance with a considerable body of evidence cited earlier. Cox *et al.* (1931) obtained a 15% reduction in blood phosphorus of guinea pigs fed

a ration containing 1,350 ppm of soluble aluminum. The phosphorus in the bones of these animals was reduced to 70% of normal in 12 weeks. No satisfactory explanation for the discrepancy can be offered on the basis of the present data. However, it is of interest to note that all reported work showing a lowering of phosphorus availability by aluminum has been conducted on non-ruminant animals. Struthers and Seiling (1950) have discussed the effects of organic anions upon phosphate precipitation. In the absence of such anions, aluminum phosphate was precipitated over the pH range of 4.0–9.0. However, when such anions, particularly of the simpler organic acids, were present, the aluminum was complexed and was not precipitated as the phosphate at the above pH range. In view of the considerable quantities of organic acids in the rumen, it is not unreasonable to suggest that the same conditions for the precipitation of phosphorus may not exist in the ruminant as in the non-ruminant. Certainly, little evidence has been obtained in the present investigation to suggest that the incorporation of aluminum sulphate in rations as a fluorine alleviator would seriously interfere with calcium or phosphorus retention and metabolism.

Summary

Concurrent chemical and radiochemical blood and balance studies were carried out with 12 lambs in four trials on rations containing 0, 0.5 and 1.0% of aluminum sulfate and of zinc sulfate. The results of radiocalcium and radiophosphorus behavior after oral and/or intravenous administration indicated little effects of increased dietary levels of aluminum or zinc. However, net retention and apparent and true digestibility of dietary calcium were markedly decreased at all levels of zinc feeding. Fecal endogenous calcium averaged 39.9 ± 1.8 mg./kg/day as compared with 28.1 ± 1.8 and 28.2 ± 1.8 mg., respectively, for those receiving the basal and aluminum-containing rations. Evidence is presented indicating that zinc may also affect the gastro-intestinal absorption of calcium. The effects of aluminum upon calcium behavior was without significance.

Phosphorus retention was likewise affected by dietary zinc, but not by aluminum. No dietary treatment effected any significant difference in fecal endogenous phosphorus loss, and it was concluded that any decreased retention of phosphorus was due to interference in absorption from the tract. These negligible effects of dietary aluminum upon phosphorus metabolism in the ruminant are discussed in light of previous studies and possible specie differences in absorption.

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THE EFFECT OF TRANQUILIZERS, DIETHYLSTILBESTROL AND OXYTETRACYCLINE ALONE AND IN COMBINATION ON PERFORMANCE OF STEERS

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IT is well known that growth rate of animals is influenced by levels or balance of macronutrients (protein, carbohydrate, fat and certain minerals) and of micronutrients (vitamins, unidentified growth factors and trace minerals). In recent years certain non-nutrients such as antibiotics and hormones have been intensively investigated and have proven capable of enhancing growth rate. Considerable nutrition research has been carried out in these laboratories to determine if tranquilizing drugs, which are used in human medicine to alleviate anxiety or tension through their action on certain nerve centers, could influence growth rate of animals.

A report of early trials by Sherman *et al.* (1958) presented evidence that growth rate and feed efficiency in cattle and lambs were enhanced by hydroxyzine [1] and *Rauwolfia vomitoria* root. The magnitude of the response appeared to be related to the quantity fed. In some of these trials the tranquilizers were fed in the presence of the antibiotic oxytetracycline [2] and/or stilbestrol and in other trials the tranquilizers were fed to animals with no antibiotic or hormone treatment.

This paper reports detailed results of three cattle trials designed to evaluate tranquilizing drugs and to investigate possible interactions between tranquilizers and hormones or antibiotics. A preliminary report of two of the present cattle trials and of two lamb trials was presented earlier (Sherman *et al.*, 1957).

Experimental Procedure

Experiment I. Thirty yearling Hereford steers were divided into three comparable lots of ten each on basis of initial weight (approximately 750 lb.). They were given a supplement and ground ear corn and were brought to a full feed as quickly as possible. The ration shown in table 1 was used throughout a 111-day fattening period. Oxytetracycline and stilbestrol were included in the protein supplement of all three lots to supply daily intakes of 80 and 10 mg. per head, respectively. Lot 1 served as control; Lot 2 received *Rauwolfia vomitoria* (0.24% reserpine equivalent), 25 mg. per head per day; and Lot 3 received hydroxyzine, 2.4 mg. per head per day. Alfalfa hay was fed once a day in a separate feeder and the protein supplement twice a day in equal portions on top of the ground ear corn which was available to the animals at all times.

[1] Trademark of Chas. Pfizer & Co., Inc for hydroxyzine is Tran-Q.
[2] Trademark of Chas. Pfizer & Co., Inc. for oxytetracycline is Terramycin.

Experiment II. Forty-eight yearling Hereford steers were divided into six uniform lots of eight each on the basis of initial weight (approximately 740 lb.). They were brought up to a full feed of ground ear corn gradually by feeding 5 lb. per head per day for the first 28 days, 10 lb. during the second 28 days and 15 lb. during the third 28-day period in addition to the protein supplement, hay and self-fed ground corncobs. After 84 days the ground corncobs were removed and the animals were self-fed ground ear corn for the balance of the trial in addition to the other ration

TABLE 1. DAILY BASAL RATIONS[a]—EXPERIMENTS I, II AND III

	Experiment I	Experiment II				Experiment III	
Consumption	0–111 days	0–28 days	29–56 days	57–84 days	85–168 days	0–84 days	85–168 days
Ground corncobs	....	Self-fed	Self-fed	Self-fed	....	Self-fed	....
Ground ear corn, lb.	Self-fed	5	10	15	Self-fed	4	Self-fed
Protein supplement, lb.	3	2	2	2	2	3	3
Alfalfa hay, lb.	3	3	3	3	3	...	2

Supplement components	Experiment I	Experiment II All periods	Experiment III All periods
	%	%	%
Soybean oil meal, 44% protein	58.67	80.05	62.00
Dehydrated alfalfa meal	20.00	.....	20.00
Dried molasses	10.00	10.00	10.00
Urea	5.00	5.00	2.00
Dicalcium phosphate	2.00	4.50	2.00
Ground limestone	1.70	....	1.70
Trace-mineralized salt	2.25	....	2.25
Trace-mineral premix	....	0.40	....
Stabilized vitamin A supplement[b]	0.05	0.05	0.05
Oxytetracycline-stilbestrol premix[c]	0.33	....	....

[a] Trace-mineralized salt and water, *ad libitum*.
[b] Gelatin-stabilized, 10,000 I.U. per lb.
[c] Contains 8 gm. oxytetracycline and 1 gm. stilbestrol per lb.

constituents given in table 1. The following treatments were employed: Lots 1 and 2, control; Lot 3, stilbestrol implant, 36 mg.; Lot 4, oxytetracycline, 75 mg.; Lots 5 and 6, hydroxyzine, 1.25 and 10 mg., respectively, per head per day in the protein supplement.

Experiment III. One hundred twenty-eight yearling Hereford steers were divided into 16 uniform lots of 8 each on the basis of initial weight (approximately 665 lb.). During the first 84 days the animals were given a high roughage ration consisting of ground corncobs self-fed and limited corn and protein supplement, as shown in table 1. During the last 84 days they received a full feed of ground ear corn and limited hay and protein supplement. The design of the experiment was a 4 x 4 factorial with one combination treatment missing in order to replicate the negative control. The plan of this experiment is shown in table 2.

Data were tested for statistical significance by Snedecor's Analysis of Variance (1946).

TABLE 2. PLAN OF EXPERIMENT III

Control (replicated)	Oxytetracycline 80 mg./day	Stilbestrol implant 36 mg.	Oxytetracycline + Stilbestrol
Hydroxyzine 2.4 mg./day	Hydroxyzine + Oxytetracycline	Hydroxyzine + Stilbestrol	Hydroxyzine + Oxytetracycline + Stilbestrol
Rauwolfia 25 mg./day	Rauwolfia + Oxytetracycline	Rauwolfia + Stilbestrol	Rauwolfia + Oxytetracycline + Stilbestrol
Reserpine 60 mcg./day	Reserpine + Oxytetracycline	—	Reserpine + Oxytetracycline + Stilbestrol

Results

Experiment I. The growth, feed consumption, feed efficiency and slaughter data are presented in table 3. Both Rauwolfia and hydroxyzine improved gains of steers in the presence of oral stilbestrol and oxytetracycline and the response to Rauwolfia was statistically significant

TABLE 3. EXPERIMENT I. EFFECTS OF TRANQUILIZERS ON PERFORMANCE OF STEERS FED STILBESTROL AND OXYTETRACYCLINE [a] (111 DAYS)

Lot number	1	2	3
Treatment	None	Rauwolfia vomitoria 25 mg.	Hydroxyzine 2.4 mg.
Number of steers	10	10	10
Weight, initial, lb.	751	746	752
Weight, 111 days	1068	1106	1099
Av. daily gain, lb.	2.86	3.24*	3.13
Av. daily feed consumed, lb.			
Ground ear corn	18.68	20.92	19.52
Supplement	2.94	2.93	2.93
Hay	3.05	2.93	3.06
Lb. feed per lb. gain	8.57	8.24	8.16
Shrink, farm to pre-kill, %	5.90	6.24	6.05
Dressing percent [b]	59.2	59.9	58.9
Carcass grade [c]	9.7	11.6*	10.1

[a] All treatment groups were fed stilbestrol, 10 mg. and oxytetracycline, 80 mg. per head per day.
[b] Dressing percent based on cold carcass and yard weights.
[c] Carcass grade numerical rating: 10, low choice; 11, average choice.
* Difference from control in average daily gain and carcass grade was statistically significant ($P<.05$).

($P<0.05$). Both tranquilizer treatments improved feed efficiency; the feed efficiency with hydroxyzine was better than that with Rauwolfia. Differences in shrink and dressing percentages were small and were not statistically significant. Carcass grade was apparently improved by both tranquilizers and was significantly different from the control in the Rauwolfia fed steers ($P<0.05$).

TABLE 4. EXPERIMENT II. EFFECT OF IMPLANTED STILBESTROL, ORAL OXYTETRACYCLINE AND ORAL HYDROXYZINE ON PERFORMANCE OF STEERS (168 DAYS)

Lot no.	1 & 2	3	4	5	6
		Stilbestrol implant	Oxytetra-cycline	Hydroxyzine	
Treatment	None	36 mg.	75 mg.	1.25 mg.	10 mg.
Number of steers	16	8	8	8	8
Weight, initial, lb.	729	733	752	738	740
Weight, 84 days, lb.	888	943	931	916	902
Weight, 168 days, lb.	1064	1132	1109	1093	1091
Av. daily gain, 84 days	1.89	2.50	2.13	2.12	1.93
Av. daily gain, 168 days	1.99	2.38*	2.12	2.11	2.09
Av. daily feed consumed, lb. 0–84 days					
Ground corncobs	6.42	7.12	8.30	6.19	6.21
Ground ear corn	10.39	10.39	10.39	10.39	10.39
Supplement	2.00	2.00	2.00	2.00	2.00
Hay	2.91	2.91	2.91	2.91	2.91
Av daily feed consumed, lb., 84–168 days					
Ground corncobs	.26	.30	.30	.22	.30
Ground ear corn	20.18	21.38	21.31	20.42	21.20
Supplement	2.02	2.02	2.02	2.02	2.02
Hay	2.02	2.02	2.02	2.02	2.02
Lb. feed per lb. gain, 84 days	11.49	8.98	11.09	10.13	11.13
Lb. feed per lb. gain, 168 days	11.60	10.12	11.61	10.94	11.24
Shrink, farm to pre-kill, %	4.80	4.31	4.46	3.95	4.94
Dressing percent [a]	60.3	60.9	60.4	61.2*	60.5
Carcass grade [b]	9.82	9.62	9.75	10.50*	10.00

[a] Dressing percent based on cold carcass and yard weights.
[b] Carcass grade numerical rating; 9, high good; 10, low choice.
* Difference from control statistically significant ($P<.05$).

Experiment II. The feedlot performance and carcass data are given in table 4. All supplements improved the gains in the first 84 days as well as in the entire 168-day period. The best response was with the stilbestrol implant which was significantly superior to the control ($P<0.05$). The oxytetracycline and both levels of hydroxyzine produced approximately equal growth responses of 5% to 6% over the control. The stilbestrol implant and both levels of hydroxyzine improved feed efficiency. Dressing percent was not affected by the hormone or antibiotic treatments but was significantly improved by the 1.25 mg. level of hydroxyzine ($P<0.05$).

Carcass grade was also significantly improved by this level of hydroxyzine; the other treatments showed only minor effects.

Experiment III. The weight, feed consumption and feed efficiency data for the high-roughage feeding period of 0–84 days and for the entire 168-day period, as well as the slaughter data, are given in table 5.

Gain and feed efficiency to 84 days were improved by oxytetracycline in all four treatments with no stilbestrol present; in the presence of stilbestrol

TABLE 5. EXPERIMENT III. EFFECTS OF TRANQUILIZERS ALONE AND IN BESTROL ON PERFORMANCE

Lot no.	1 & 2	3	4	5	6
Item	None	Oxytetracycline [a]	Stilbestrol [b]	Oxytetracycline+ stilbestrol	Hydroxyzine [c]
Number of steers	16	8	8	8	8
Weight, initial, lb.	664	666	664	664	665
Weight, 84 days	808	816	818	824	812
Weight, 168 days	1025	1004	1068	1087	1041
Av. daily gain, 84 days	1.72	1.79	1.84	1.91	1.75
Av. daily gain, 168 days	2.13	2.02	2.40*	2.52*	2.24
Pooled tranquilizer treatments, 84 days		1.81			
Pooled tranquilizer treatments, 168 days		2.27			
Av. daily feed, lb., 0–84 days					
Ground ear corn	3.68	3.60	3.60	3.60	3.76
Protein supplement	3.03	3.10	3.11	3.11	2.95
Ground corncobs	13.57	13.62	13.97	13.81	13.78
Av. daily feed, lb., 85–168 days					
Ground ear corn	22.77	21.98	24.23	23.76	23.14
Protein supplement	2.95	2.95	2.97	2.96	2.95
Hay	.57	.57	.57	.57	.57
Lb. feed per lb. gain, 84 days	11.78	11.26	11.72	10.75	11.66
Lb. feed per lb. gain, 168 days	10.96	11.36	10.08	9.50	10.53
Pooled tranquilizer treatments, 84 days		11.38			
Pooled tranquilizer treatments, 168 days		10.48			
Shrink, farm to pre-kill, %	5.20	5.14	4.54	4.89	5.42
Cooler shrink, %	1.66	1.91	1.60	2.02	1.97
Dressing percent [f]	58.9	59.8	59.3	58.7	58.0
Carcass grade [g]	8.56	8.25	7.50	8.38	8.50

[a] Oxytetracycline, 80 mg. per head per day.
[b] One initial implant of 36 mg. stilbestrol.
[c] Hydroxyzine, 2.4 mg. per head per day.
[d] *Rauwolfia vomitoria*, 25 mg. per head per day.
[e] Reserpine, 60 mcg. per head per day.

implants, the antibiotic enhanced gain in all of the three treatments and decreased feed required per lb. gain in two of the three lots where comparisons are possible. Oxytetracycline improved gains and feed efficiency at 168 days in three of the four lots without stilbestrol and in all of the stilbestrol-implanted treatments.

Stilbestrol implant improved gains in all treatments at 84 days and at 168 days with significance ($P<0.05$) in only one treatment at 84 days and in all treatments at 168 days, as compared with the negative control.

At 84 days, gains and feed efficiency of all four lots receiving hydrox-

yzine, two of the four lots receiving *Rauwolfia vomitoria* and all three lots receiving reserpine were superior to the corresponding control lots. At 168 days gains of all four lots and feed efficiency of three of the four lots receiving hydroxyzine were superior to the values for the corresponding controls; two of the four lots receiving Rauwolfia and two of the three lots receiving reserpine were superior to the corresponding control lots in gain and feed efficiency. The data on the different groups within each

COMBINATION WITH ORAL OXYTETRACYCLINE AND IMPLANTED STIL- OF STEERS (168 DAYS)

7	8	9	10	11	12	13	14	15	16
Hydroxy-zine+ oxytetra-cycline	Hydroxy-zine+ stilbes-trol	Hydroxy-zine+ oxytetra-cycline+ stilbes-trol	Rau-wolfia[d]	Rau-wolfia+ oxytetra-cycline	Rau-wolfia+ stilbes-trol	Rauwolfia + oxytetra-cycline+ stilbes-trol	Reser-pine*	Reserpine + oxytetra-cycline	Reserpine + oxytetra-cycline+ stilbes-trol
8	8	8	8	8	8	8	8	8	8
665	664	664	665	664	665	665	665	665	665
828	845	849	799	813	838	846	813	837	843
1056	1083	1096	1023	1040	1081	1085	1048	1079	1081
1.95	2.16*	2.19*	1.60	1.77	2.05	2.15*	1.77	2.05	2.24*
2.33	2.49*	2.57*	2.13	2.24	2.47*	2.48*	2.28	2.47*	2.48*
2.01†				1.89				2.02†	
2.41†				2.33				2.41†	
3.76	3.76	3.76	3.76	3.76	3.76	3.76	3.76	3.76	3.76
2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95
13.56	13.90	14.51	14.02	13.71	14.64	14.43	13.31	14.02	14.40
22.19	24.00	24.39	23.24	23.56	24.04	23.81	23.04	23.60	24.31
2.95	2.96	2.96	2.95	2.96	2.95	2.93	2.95	2.96	2.96
.57	.57	.57	.57	.57	.57	.57	.57	.57	.57
10.41	9.57	9.68	12.94	11.55	10.40	9.83	11.31	10.12	9.93
9.86	9.66	9.55	11.13	10.63	9.89	9.81	10.21	9.70	9.89
10.33				11.18				10.34	
9.90				10.36				9.93	
4.74	4.52	4.63	5.52	4.64	5.01	4.36	4.89	5.38	3.89
2.45	2.01	2.10	1.64	1.62	1.76	2.02	1.85	2.04	1.88
59.2	58.4	58.9	58.6	60.1	59.7	60.3	58.6	58.5	59.2
8.12	8.25	7.62	8.75	8.88	8.12	7.14	8.50	8.75	8.25

[g] Dressing percent based on cold carcass and yard weights.
[e] Carcass grade numerical rating: 8, average good; 9, high good.
* Difference from negative control statistically significant ($P<0.05$).
† Difference between pooled tranquilizer treatments and pooled control treatments statistically significant ($P<0.05$).

tranquilizer treatment were combined for statistical analysis in comparison with the controls. The gains of the animals on the pooled hydroxyzine and reserpine treatments were significantly superior to the gains of the control treatments at 84 and 168 days ($P<0.05$). Feed efficiency values of the pooled hydroxyzine treatments and the reserpine treatments were better than the corresponding feed efficiency value of the pooled control treatments.

Differences in shrink, carcass grade and dressing percent between lots were small and were not statistically significant. In general, the grades of

the animals in this experiment were not as good as in previous trials owing to the relatively small amount of corn which they received.

Discussion

In three cattle trials hydroxyzine produced growth increments of 6% to 12% and feed efficiency improvement of 4.8% to 9.2%. *Rauwolfia vomitoria* induced growth and feed efficiency responses in two trials; the magnitudes of the responses were somewhat more variable between trials than with hydroxyzine. In the one trial in which crystalline reserpine was fed, responses were similar to those with hydroxyzine and were slightly better than with *Rauwolfia vomitoria*. Since the 60 mcg. level of reserpine fed was equivalent to the reserpine furnished by 25 mg. *Rauwolfia vomitoria* based on chemical analysis, the data suggest that the beneficial effects of *Rauwolfia vomitoria* are entirely attributable to its reserpine content. Further research is necessary, however, to establish this point.

There was no evidence of downgrading of carcasses due to tranquilizers in any of the experiments and in two trials there was a significant improvement. Performance was improved by tranquilizers in the presence of oral stilbestrol and oxytetracycline in Experiment I, in the absence of hormone and antibiotic in Experiment II, and appeared to be additive to hormone or antibiotic responses in Experiment III, a trial controlled for interactions. In a report of previous trials by Sherman *et al.* (1958) tranquilizers improved performance in one trial in the presence of stilbestrol implants and in another trial in the absence of hormone or antibiotic. The results to date with cattle indicate that improved performance may be expected from tranquilizers fed at proper levels in the presence or absence of hormone or antibiotic treatment. Additional research is indicated to further investigate possible interactions between tranquilizers and hormones, antibiotics and various dietary factors.

Roughage to concentrate ratio did not appear to influence the response to tranquilizers in these trials. In Experiment III a high level of roughage was fed continuously during the first half and a high concentrate level during the last half of a 168-day feeding period; the responses to tranquilizers were of approximately the same order of magnitude at 84 days as at 168 days. Similar results were obtained in Experiment II in which cattle were started on a high roughage ration which was gradually changed to a high concentrate ration duing the first 84 days.

The levels of tranquilizers fed in these experiments were considerably lower than the levels prescribed in human therapy for control of nervous tension. There was no evidence of sedation as judged by general behavior, ease of handling, or resting habits of the cattle. Further evidence that the drugs did not produce marked sedation was apparent in the feed consumption data showing that growth responses were in most instances associated with increased feed consumption. It is entirely possible, however, that the action of the low levels of tranquilizers which induced growth responses

in these experiments could have been mediated through mild effects on certain nerve centers to exert a protective effect against environmental stress without producing measurable sedation.

In both trials with oxytetracycline in which comparisons were possible (Experiments II and III) growth and feed efficiency were enhanced without alteration of carcass quality. Similar results from feeding this antibiotic to cattle with or without stilbestrol have been previously reported by other workers including Dyer *et al.* (1957), Kolari and Harvey (1957) and Adams *et al.* (1954).

Stilbestrol implants (36 mg.) produced outstanding growth and feed efficiency responses in both experiments in which they were evaluated and only slight inconsistent tendencies toward downgrading. The subject has been adequately reviewed in recent publications by Perry *et al.* (1958) and Clegg *et al.* (1957).

Summary

The tranquilizing drug hydroxyzine, which was evaluated at low levels alone or in combination with stilbestrol or oxytetracycline in three steer-fattening experiments produced increases ranging from 6 to 12% in average daily gain and improvement of 4.8 to 9.2% in feed efficiency. Ground *Rauwolfia vomitoria* root produced smaller growth and feed efficiency responses in one trial and 11.3% and 3.5%, respectively, in another. Reserpine tested in one trial enhanced average daily gain by 6.2% and feed efficiency by 5.2%. There was no indication of lowered carcass quality and in several instances the tranquilizers appeared to improve carcass grade. The use of nutritional levels of tranquilizers was not accompanied by gross evidence of sedation.

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INFLUENCE OF FISTULATION ON THE DIGESTIBILITY OF FEEDS BY STEERS[1]

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IN studying the digestibility of various rations, Haynes *et al.* (1955) obtained an average total digestible nutrient value of 55.6 with fistulated steers as compared with 62.2 with milking cows fed identical rations. The steers were fed at a lower level of intake than the cows, which was expected to favor higher digestibility by the steers. In attempting to explain these results, it appeared possible that the presence of the fistulas may have depressed digestibility, especially since the fistulas were opened several times each week to permit a simultaneous study of the digestion of cotton threads suspended in the rumen. The wide use of fistulated animals in nutrition studies made it desirable to obtain an anwer to the question as to whether or not fistulation influenced the digestibility of a ration.

A study was planned, therefore, to compare the digestibility of different kinds of rations by fistulated and intact steers. In order to obtain data under widely varying conditions, tests were made with (1) hay alone, (2) concentrates alone, and (3) hay plus concentrates, equal parts.

Several workers have fed concentrates alone to ruminants and obtained reasonable digestion coefficients (Ewing *et al.*, 1915, 1917; Forbes *et al.*, 1931; Mead and Goss, 1935; Watson *et al.*, 1947).

Experimental

Six Holstein steers weighing approximately 400 lb. were paired in such a manner that variation in size within pairs was smallest. A rumen fistula was introduced in one of each pair chosen at random. Pressure plugs described by Balch and Johnson (1948) were used through the first period and pneumatic plugs described by Dougherty (1955) through the second and third periods. In order to permit air to enter the rumen the plugs were removed at intervals of 3 to 4 days for 5–10 minutes. In no case were the plugs completely airtight, though the latter type, after being somewhat modified, served well to retain the rumen ingesta.

The design was a 3 x 3 Latin square employing three rations, three pairs of steers and three periods. Since one steer of each pair was fistulated, the design can also be regarded as two simultaneous 3 x 3 Latin squares, one with intact and the other with fistulated steers. Hence, 18 single

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TABLE 1. PROXIMATE ANALYSIS AND HEAT OF COMBUSTION OF FEEDS ON DRY MATTER BASIS

Feed	Protein (6.25 x N)	Crude fiber	N-free extract	Ether extract	Ash	Heat of combustion
	%	%	%	%	%	kcal./gm.
Concentrates [a]	28.22	12.07	47.49	6.63	5.59	5.13
Alfalfa hay	19.73	28.60	42.95	1.66	7.06	4.59

[a] Composed of corn distillers dried grains plus 1.5% of ground limestone.

digestion trials were conducted. The duration of preliminary periods was 20 days, except for one single trial in which it was 14 days. Total collections were carried out for 10 days in each trial. Ten-percent aliquots were air dried daily at about 70° C for 24 hours and pooled. The 10-day composite samples of each single trial were weighed, ground in a Wiley mill, sub-sampled and stored in airtight jars.

Daily feed allotments were: (1) 4.25 kg. alfalfa hay, (2) 2.47 kg. concentrates and (3) 2.11 kg. alfalfa hay plus 1.25 kg. of concentrates. These allotments supplied 75% of the lower values of TDN recommended in Morrison's Feeding standards. In the mixed ration each feed was planned to supply one half of the TDN. Ground limestone at a level of 1.5% was added to all the concentrate ration. The animals were fed twice a day; consumption was complete throughout the trial. The design made it possible to test the existence of associative (non-additive) effects of the feeds.

Proximate analysis (A.O.A.C., 1945) was carried out on all the samples of feeds and feces. Combustible energy was determined in a Parr oxygen bomb calorimeter with an adiabatic jacket. The results are reported in table 1.

The data were analyzed as a set of two simultaneous 3 x 3 Latin squares with a total of 17 degrees of freedom for each of six sets of individual

TABLE 2. EFFECT OF RUMEN FISTULATION ON DIGESTION COEFFICIENTS

Fraction	Digestion coefficients		
	Intact [a]	Fistulated [a]	Pooled [b]
Protein	77.4	76.5	77.0 ± 1.6
Crude fiber	45.4	45.2	45.3 ± 2.4
N-free extract	73.5	73.4	73.5 ± 1.6
Ether extract	61.4	61.9	61.7 ± 3.0
Organic matter	67.5	67.2	67.4 ± 1.5
Energy	66.2	65.6	65.9 ± 1.6

[a] Each value is an average of 9 observations.
[b] Each value is an average of 18 observations.

TABLE 3. COMPARISON OF DIGESTION COEFFICIENTS CALCULATED AS WEIGHTED MEANS TO COEFFICIENTS ACTUALLY DETERMINED FOR COMBINED HAY AND CONCENTRATES

Fraction	Weighted mean coefficients [a]	Determined coefficients
Protein	77.3	76.1 ± 1.6
Crude fiber	41.0	39.9 ± 2.2
N-free extract	73.4	73.8 ± 1.7
Ether extract	70.1	69.5 ± 2.2
Organic matter	65.5	66.1 ± 1.6
Energy	65.1	64.8 ± 0.7

[a] From separate trials of hay alone and concentrate alone.

digestion coefficients: Protein, ether extract, crude fiber, N-free extract, organic matter and energy (i.e., heat of combustion).

Results

The differences between the mean digestion coefficients of intact and fistulated steers (table 2) are not significant at the $P = 0.05$ level, showing that fistulation did not alter the digestibility of the feeds. The interactions between rations and fistulation treatment were not significant.

The mean digestion coefficients obtained with six animals on the mixed ration of hay plus concentrates are compared in table 3 with values calculated as weighted means of the coefficients for the all-hay and all-concentrate ration. The two sets of coefficients agree closely and, since the differences are not significant the results demonstrate the lack of any "associative" effects between the hay and the concentrates.

Since interest in the use of digestible energy in feed evaluation is on the increase, the TDN values, the digestible energy values, the conversion factors from TDN percent to digestible energy expressed in therms per pound for each ration used in this experiment and the digestible protein values of the feeds are presented in table 4.

TABLE 4. TDN-DIGESTIBLE ENERGY RELATIONSHIP AND DIGESTIBLE PROTEIN VALUES OF THE FEEDS ON AN AIR DRY (AS FED) BASIS

Feed	TDN	Digestible energy	Conversion factor	Digestible protein
			therms/lb. / TDN percent	
	%	therms/lb.		%
Concentrate	71.9	1.597	0.02221	20.6
Alfalfa hay	51.9	1.097	.02114	17.6
Mixed ration	58.4	1.262	.02161	—

Discussion

Non-additive (or "associative") digestibility was first described over a hundred years ago. In 1855 Ritthausen and Scheven (as cited by Haubner and Hofmeister, 1864) found that starch and soluble carbohydrates depressed the digestibility of crude fiber. Henneberg and Stohmann (1863) reported increased digestibility when poor hay was supplemented with protein. Feeding trials with large amounts of feeds like potatoes and beets, and the tendency to raise excessively fat stock brought about a wealth of data on this subject. Two unrelated effects are best documented: (a) The apparent decrease in nitrogen digestibility with decreasing levels of nitrogen intake resulting from an increasing error introduced by metabolic nitrogen, and (b) the well-known depression of cellulose or crude fiber digestion with increasing levels of starch or soluble carbohydrate. On extremely low intakes of nitrogen a general depression of digestibility occurs probably because of a nitrogen deficiency of the microflora in the rumen, as evidenced by bacterial counts (Burroughs *et al.*, 1950; Moir and Williams, 1950). Such high-energy, low-nitrogen rations are unbalanced and unpractical today and are not in common use.

Forbes *et al.* (1931) found the metabolizable energy of corn meal unchanged when fed exclusively or with alfalfa hay. Watson *et al.* (1939, 1941, 1947) in a series of studies showed that non-additive effects, if any, are negligible in present-day practical rations. The data presented in this paper support the contention that digestion coefficients, determined on adequate rations are additive.

Summary

Eighteen single digestion trials were conducted with three intact and three rumen-fistulated steers fed three rations: (1) all hay, (2) all concentrate and (3) hay plus concentrate. Two simultaneous 3 x 3 Latin squares were employed.

The differences between digestion coefficients obtained with intact and fistulated steers were not significant at the P=0.05 level. Non-additive digestibility was not apparent; the digestion coefficients of the mixed ration (3) were in good agreement with those computed for this ration from the single-feed rations (1) and (2).

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SOME CARCASS CHARACTERISTICS OF SWINE IN THE EIGHTH GENERATION OF PRODUCTION UNDER FOUR COMBINATIONS OF FULL AND LIMITED FEEDING [1, 2, 3]

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CARCASS quality and meatiness are two of the major productive traits which have a pronounced effect on economic returns in swine production. These carcass traits are the result of the interaction of the animal's genetic capabilities with its environment. The plane of nutrition on which the animal is raised represents a major part of the environmental effect. The genotype of an animal impels it to follow a particular line of development; however, if the ration received varies in amount, differing patterns of development may result even when the hereditary make-up is exactly the same, as in identical twins.

McMeekan (1940) found that carcasses of barrows and gilts fed to 200 lb. live weight on a high plane of nutrition contained 38% fat as compared with 27% for those fed under a low plane of nutrition. Moreover, the high-plane group had less bone (11% versus 12%) and muscle (40% versus 49%) than the low-plane group.

McMeekan and Hammond (1940) reported that different planes of nutrition during different periods in the development of swine affected the type and quality of the carcass. Rapid early growth and slow later growth produced the best development of muscle and a minimum of fat.

Brugman (1950) reported that the carcass qualities of swine can be changed by limiting the feed intake to 70% of full feed up to 150 lb. and full feeding from then until the animals reach approximately 220 lb. live weight. "Low-high" pigs produced a significantly higher percentage of total trimmed primal cuts and a lower percentage of total lard than the "high-high" pigs.

Whatley and co-workers (1953) found that pigs fed a low-energy ration from 140 to 225 lb. produced leaner carcasses than pigs receiving a high

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energy ration, as indicated by less backfat, higher carcass specific gravity, and larger areas of loin and ham.

Lucas and Calder (1956) reported that restriction of the plane of nutrition after 100 lb. live weight markedly reduced shoulder-fat measurements of gilts, but did not affect the depth of shoulder fat of barrows.

The present study was undertaken to study the carcass characteristics of swine produced for eight generations under four combinations of full and limited feeding. The study was designed to reveal some of the carcass characteristics of swine produced under full and limited feeding, and also to evaluate the results upon carcass characteristics when swine are selected under one plane of nutrition and their progeny are fed under a different plane of nutrition.

Material and Methods

The data in this study were secured from the eighth generation of swine on an experiment started in 1947 to study the relationship between nutrition and the improvement of animals for meat production through breeding.

The foundation stock for the project was created by crossing five Danish Landrace boars with five relatively unrelated groups of five Chester White sows each. The progeny were divided equally and at random, at 56 days of age, into two lines which could be expected to be genetically similar. One line, high-plane, was full fed (self-fed) from weaning to 150 lb. The other line, low-plane, was fed 70% as much as was eaten by the high plane pigs at comparable weights up to 150 lb. From weights of 150 lb. until farrowing, all pigs were hand-fed to prevent the high-plane pigs from becoming obese. High-plane animals received sufficient feed to permit an average daily gain of 1.5 to 2.0 lb. The low-plane pigs received 70% of this amount. After farrowing, the high-plane sows were returned to full feed, and the low-plane sows received 70% of full feed.

At 150 lb. the animals were indexed, taking into account prolificacy and rate of gain, and the highest indexed animals were saved to produce the next generation. Although detailed body measurements were secured on all animals at 150 lb. live weight, no selection pressure was applied for type or conformation. These measurements were obtained to provide data on any correlated response in body type that might accompany the direct selection for prolificacy and rate of gain. (These findings are to be reported in a later paper.) The lines were closed to outside blood; however, inbreeding was kept at a minimum by systematic crossing of the groups within lines, with no mating closer than between quadruple second cousins. The same system of matings was practiced in each line so as to keep the inbreeding coefficients as nearly alike as possible.

In the sixth generation, half of each line was shifted to the other plane of nutrition, thus creating four lines: (1) high-high (H.H.) raised under six generations of full-feeding and remaining on full-feeding; (2) high-low

(H.L.) raised under six generations of full-feeding and then shifted to limited feeding; (3) low-high (L.H.) raised under six generations of limited feeding and then shifted to full-feeding; (4) low-low (L.L.), raised under six generations of limited feeding and remaining on limited feeding.

The composition of the rations fed to each line was identical; the only difference was the level at which the animals were fed (table 1). The rations were calculated to provide adequate protein, minerals, and vitamins at the levels fed to low-plane pigs, the only deficiency being energy. Thus, high-plane pigs received adequate energy but excesses of protein, minerals, and

TABLE 1. RATIONS FED

Ingredients	Weaning to 75 lb.	76 lb. to 150 lb. and lactating sows	Pigs over 150 lb. and gestating sows
	lb.	lb.	lb.
Ground barley	32.0	30.0	31.0
Ground wheat	22.0	30.0	24.0
Mill run	0.8	0.7	0.7
Corn distillers dried solubles	0.7	0.6	0.6
Soybean meal	17.0	14.0	14.0
Fish meal	11.0	9.0	9.0
Dried skim milk	5.0	4.0	4.0
Dehydrated alfalfa meal	9.5	10.5	15.0
Ground oyster shell	0.7	0.6	0.6
Steamed bone meal	0.7	0.6	0.6
Iodized salt	0.6	0.5	0.5
Mineral mix[a]	0.08	0.08	0.08

[a] The mineral mix was made up of the following: $ZnCO_3$, 50 gm.; $CuSO_4$, 150 gm.; Fe_2O_3, 200 gm.; $FeSO_4$, 500 gm. $MnSO_4$, 200 gm.; plus irradiated yeast type 9F, 1820 gm., and soybean oil meal, 2500 gm.

vitamins. All animals were maintained in cement dry lots throughout the study.

Thirty-two gilts from the eighth generation were fed to weights of approximately 215 lb. on their respective planes of nutrition and were slaughtered to secure the data reported herein. There were eight gilts from each line. Thus, two groups of gilts were included that had been selected and fed under eight generations of full or limited feeding (H.H. and L. L., respectively). The other two groups of gilts were selected for six generations under one plane of nutrition and then fed for two generations on the other plane of nutrition (H.L. and L.H.). Only gilts were used to avoid any error owing to sex differences. Barrows are reported to carry less bone and muscle and more fat than gilts (McMeekan, 1940). Gilts yield a higher percentage of lean cuts (Whatley *et al.*, 1953).

All pigs were slaughtered as near 215 lb. live, shrunk weight as possible, with a range in weight of 205 to 223 lb. The gilts were removed from feed 24 hours before slaughter but were allowed free access to water. All animals

were dressed packer style. The carcasses were given a three-day chill prior to cutting and trimming into primal cuts. All slaughtering, weighing, and cutting were performed by the senior author in order to establish uniform data for the 32 carcasses. The hams, loins, and shoulders were trimmed to allow a ¼-inch cover of fat and the bacons were all trimmed at the teat line.

Data were recorded on shrunk live weight, chilled dressed weight, yield of trimmed primal cuts, carcass length, lean loin-eye area between the tenth and eleventh rib, backfat probes on the live gilts, and carcass fatback thickness over the first and last ribs and over the last lumbar vertebra.

The carcass data were recorded for both halves of the carcass to reduce the error that might occur from unequal splitting of the carcass and to minimize the trimming error. Carcass length was measured from the lower point of the aitch bone to the anterior edge of the first rib. Loin-eye tracings were secured on both halves of each carcass. The average area of the longissimus dorsi muscle was computed from three planimeter readings for each half. Live backfat probes were secured just prior to slaughter according to the method of Hazel and Kline (1952, 1953). Carcass fatback thickness, which included the skin, was measured to the nearest one-tenth inch.

Correlations between measured characteristics, regressions of certain carcass characteristics on age and weight on a total group basis, and analysis of variances were calculated (Snedecor, 1946).

Results

Slaughter Weights and Ages. The average weight at slaughter for full-fed gilts (H.H. and L.H.) was 216.2 lb. as compared with 213.9 lb. for gilts receiving 70% of full feed (H.L. and L.L.). The average slaughter weight for gilts from a selection background of full-feeding (H.H. and H.L.) was 215.6 lb. as compared with 214.6 lb. for gilts from a selection background of limited feeding (L.H. and L.L.). The data were not adjusted for these slight variations in live slaughter weight, because the regressions of carcass characteristics on live weight indicated that variation in weight had little effect on the various carcass characteristics (table 4).

The average age at slaughter for full-fed (H.H. and L.H.) and limited-fed gilts (H.L. and L.L.) were 211.63 and 268.13 days, respectively. The approximately 7 months required for the full-fed gilts to reach market weight is explained by the fact that the 32 top-gaining gilts from the high-plane were retained to produce the ninth generation. Because of the highly significant difference of 56.5 days, the data for fatback thickness, loin-eye area, and yield of trimmed primal cuts were adjusted to the mean of 239.9 days by use of regression coefficients (table 4).

Effects of Plane of Nutrition. On the basis of the unadjusted data, the full-fed gilts averaged 1.42 inches of carcass fatback, as compared with 1.10 inches for gilts receiving 70% of full feed (table 2). This difference

of 0.32 inch was significant at the 0.01 level of probability. When adjusted to a constant age, the difference in fatback thickness increased to 0.56 inch (P=0.01).

Full-fed and limited fed gilts had average loin-eye areas of 3.86 and 3.97 sq. in., respectively, before adjustment of the data to a constant age. The difference of 0.11 sq. in. in favor of the limited-fed gilts was not statistically significant. When adjusted to a constant age, however, the loin-eye areas of the full-fed gilts exceeded those for limited-fed gilts by

TABLE 2. SUMMARY OF THE AVERAGES OF CARCASS CHARACTERISTICS WITHIN GROUPS

Treatment	Number of gilts in treatment	Slaughter age	Primal cut yield	Adjusted[a] primal cut yield	Lean loin-eye area	Adjusted[a] lean loin-eye area	Fat-back thick-ness	Adjusted[a] fat-back thick-ness
		da.	lb.	lb.	sq. in.	sq. in.	in.	in.
High-high (H.H.)	8	214.13	102.40	103.76	4.13	4.31	1.40	1.51
Low-high (L.H.)	8	209.13	102.66	104.17	3.58	3.79	1.44	1.57
Low-low (L.L.)	8	265.50	103.92	102.57	3.68	3.51	1.04	0.93
High-low (H.L.)	8	270.75	107.83	106.19	4.25	4.04	1.16	1.03
		Averages and differences between groups						
Plane of nutrition								
Full-fed (H.H. & L.H.)	16	211.63	102.53	103.97	3.86	4.05	1.42	1.54
Limited-fed (L.L. & H.L.)	16	268.13	105.88	104.38	3.97	3.78	1.10	0.98
Difference	32	56.50**	3.35**	0.41	0.11	0.27*	0.32**	0.56**
Selection background								
Full-fed (H.H. & H.L.)	16	242.44	105.12	104.98	4.19	4.18	1.28	1.27
Limited-fed (L.L. & L.H.)	16	237.32	103.29	103.37	3.63	3.65	1.24	1.25
Difference	32	5.12	1.83	1.61	0.56**	0.53**	0.04	0.02

[a] Data adjusted to a mean constant age of 239.9 days.
* Significant at the 0.05 level of probability.
** Highly significant at the 0.01 level of probability.

0.27 sq. in. (P<0.05). Thus, the full-fed pigs were both thicker in backfat and larger in loin-eye on an adjusted basis, which means that they were bigger at the same age than the limited-fed pigs.

Carcasses from the limited-fed gilts yielded an average of 3.35 lb. more trimmed primal cuts than did the carcasses from full-fed gilts (P=0.01). Adjustment of the data to a constant age, however, reduced the difference in primal cut yield to a non-significant 0.41 lb.

Effect of Selection Background. Gilts from a selection background of full-feeding (H.H. and H.L.) did not have a significantly greater carcass fatback average than gilts from a selection background of limited feeding (L.L. and L.H.), average measurements being 1.28 inches and 1.24 inches, respectively. Upon adjustment to a constant age, the difference between the

means remained nonsignificant, and all the differences could be entirely due to chance. On the basis of these data, however, the L.H. gilts exceeded the H.H. gilts on fatback thickness on both the adjusted and unadjusted data. The same was true for H.L. gilts as compared with L.L. gilts.

Average unadjusted loin-eye area for gilts from a selection background of full feeding was 4.19 sq. in. This increase of 0.56 sq. in. over the 3.63 sq.-in. average for gilts from a selection background of limited feeding is statistically significant ($P<0.01$). When adjusted to a constant age, the difference of 0.53 sq. in. remained significant at the 0.01 level of probability. The H.L. gilts had the largest average loin eye (4.25 sq. in.) on the basis of the unadjusted data, whereas the L.H. gilts possessed the smallest average loin eye (3.58 sq. in.).

The gilts with a selection background of full feeding yielded an average of 1.83 lb. of trimmed primal cuts more per carcass than gilts from a selection background of 70% of full feeding. This difference in favor of gilts selected from a background of full feeding was reduced to 1.61 lb. when the data were adjusted by means of regression coefficient to a constant age. Neither the adjusted nor unadjusted difference in primal-cut yield was statistically significant.

On the basis of the unadjusted data, there was a significant interaction between selection background and plane of nutrition on yield of primal cuts. This interaction was a result of the size of the differences; and when the data were adjusted to a constant age, the significant interaction dropped below statistical significance.

Measurement Correlation. A highly significant positive correlation between loin-eye area and yield of primal cuts was found within groups for both the non-adjusted and the age-adjusted data ($r=0.574$ and 0.511, respectively). The gross correlation was also highly significant for both the raw and adjusted data ($r=0.569$ and 0.468, respectively).

On a within-treatment basis, carcass fatback thickness and yield of primal cuts were correlated negatively for the non-adjusted and the adjusted data ($r=-0.227$ and -0.052, respectively). Loin-eye area was also negatively correlated with carcass fatback thickness ($r=-0.267$) on a within-treatment basis. These correlations were not statistically significant.

Carcass length was not correlated to any degree with primal-cut yield ($r=0.077$) on a within-treatment basis.

The correlations between live backfat probes and carcass fatback measurement for among groups and within treatment groups were highly significant ($r=0.801$ and 0.702).

Discussion

The ideal meat-type hog should yield a maximum amount of lean cuts with the minimum amount of fat necessary to enhance the tenderness and palatability of the cuts of pork. Some authorities contend this type of

hog can be produced through controlled nutrition; others feel that the goal can be accomplished easier and more economically through a selective breeding program (Whatley *et al.*, 1953). Probably, the end product must be obtained through a combined breeding and nutritional approach.

In the current study, hogs fed 70% of full feed yielded 3.35 lb. more trimmed primal cuts ($P<0.01$) and 0.32 inch less backfat ($P<0.01$) at slaughter weights of approximately 215 lb. than did their full fed relatives. On the basis of these criteria, it appears that limited-feeding may offer a good approach to producing meat-type hogs; however, further analysis of the results reduces the optimism for the advantages to be derived from such a management procedure.

The limited-fed hogs required 56.5 days longer to reach slaughter weight than did the full-fed hogs. Apparently the greater amount of lean meat

TABLE 3. GROSS AND WITHIN-TREATMENT CORRELATIONS BETWEEN CERTAIN CHARACTERISTICS IN SLAUGHTER HOGS[a]

Item	Primal cuts	Loin-eye area	Carcass fatback	Carcass length	Live weight	Live probe
	0.546**	0.600**	−0.622**	.....	.....	.....
Age	0.298	0.412*	−0.216	.....	.....	.....
	.....	0.569**	−0.380	−0.051	0.181	.....
Primal cuts	.....	0.574**	−0.227	0.077	0.265	.....
	.....	.....	−0.167	.....	0.083	.....
Loin-eye area	.....	.....	−0.267	.....	0.095	.....
Carcass fat-	.....	.....	.....	.....	.....	0.801**
back thickness	.....	.....	.....	.....	.....	0.702**

[a] Upper figure is gross correlation and lower figure is within-treatment correlation.
* Significant at the 0.05 level of probability.
** Highly significant at the 0.01 level of probability.

produced may be the result of a longer growing period. The highly significant positive correlation between age and primal-cut yield on the unadjusted data indicates a strong age effect (table 3). Older animals yield a higher percentage of primal cuts than younger animals of the same weight when level of feeding is one of the main causes of the variance in age. On a within level of feeding basis, the correlation is not significant. When the data were adjusted to a constant age, the difference in yield of trimmed primal-cuts between the full- and limited-fed hogs was not significant statistically. Of additional consideration, the extra 2 months of labor, restriction of additional use of feeding facilities, and extended market and health risks would, under most conditions, not be properly compensated by the increased trimmed primal-cut yield obtained by the longer growing period.

Pigs with a selection background of full-feeding when fed 70% of full feed (H.L.) exceeded their full-fed relatives (H.H.) by 5.43 lb. in yield of trimmed primal cuts. On the other hand, pigs with a selection back-

ground of limited feeding (L.H.) yielded 1.26 lb. less primal cuts when placed on full feeding than did their relatives that remained on 70% of full feeding (L.L.). Selection under full feeding tended to favor selection for fuller development of primal cuts when such animals were placed on restricted feeding. In view of the lack of agreement among geneticists and animal breeders as to whether breeding stock should be selected under the conditions to which their progeny will be exposed, this type of interaction should be further investigated.

The slight advantage in area of loin eye of limited-fed gilts was lost when the data were adjusted to a constant age. The larger loin-eye area in the unadjusted data for the gilts on 70% of full feed could be attributed to their older age at slaughter, which provided a longer growth period for muscle development. The 0.27-sq. in. significant advantage of high-plane gilts in loin-eye area, when data were adjusted to a constant age, could be due to a fuller development of diameter of individual muscle fibers under a full plane of nutrition. This hypothesis is suggested by the work of McMeekan (1940).

TABLE 4. REGRESSION COEFFICIENTS FOR EFFECTS OF AGE AND WEIGHT ON CERTAIN CARCASS CHARACTERISTICS

	Effect of	
Carcass characteristic	1 day in age	1 lb. in weight
Fatback thickness	0.0042 in.	0.0004 in.
Loin-eye area	0.0069 sq. in.	0.00015 sq. in.
Primal cut yield	0.0530 lb.	0.0023 lb.

The highly significant differences in fatback thickness between full and limited-fed pigs, on both the unadjusted and adjusted data, would be expected. The reason is the higher energy intake of the full-fed pigs, which would leave more nutrients available over and above maintenance requirements for fat deposition. With a regression coefficient of 0.0042 inch of fatback for each increase of one day in age (table 4), the difference between the fatback thicknesses of the groups was increased when the data were adjusted to a constant age.

Gilts from a selection background of full feeding had a highly significant advantage in lean loin-eye area (table 2). Evidently, pigs produced from a background of full feeding had an advantage over pigs produced from a background of limited nutrition. This finding is of considerable interest because selection pressure was not exerted for type or conformation. Apparently, a correlation exists between rate of gain and muscle development. Also the genes for loin-eye development must express themselves more completely under a regimen of full feeding. Such assumptions could account for the apparent automatic selection for well muscled hogs accompanying the deliberate selection for rate of gain under full feeding.

Of additional interest are the adjusted loin-eye areas for the four groups of gilts. Arranged in descending order of loin area, they are rated H.H., H.L., L.H., and L.L. (table 2). The loin area of the pigs selected under eight generations of full feeding is considerably larger than the loin-eye area of pigs selected under eight generations of limited feeding. This gives added strength to the assumption that rapid gains are correlated with superior muscle development, and the data for muscle development support Hammond's (1947) thesis that a character can best be selected for under environmental conditions which favor its fullest expression.

The gene complex that conditions muscle development in pigs raised on full feeding appears to be identical to the genotype that conditions muscular development under restricted feeding. The environment of limited feeding, however, places a lower limit on the phenotypic expression of the genotype; full feeding permits it full expression.

Hazel and Kline (1953) found a correlation of 0.66 between the loin-eye area and the percentage of lean cuts in 23 carcasses. In the present study, the correlations between loin-eye area and primal cut yield were found to be 0.51 and 0.57 for the adjusted and non-adjusted data, respectively. These highly significant correlations indicate that the loin-eye area should be a fairly reliable indicator of the yield of primal cuts.

The strong correlation between loin-eye area and primal cut yield makes one wonder why gilts selected from a background of full-feeding had larger (highly significant) loin-eye areas than gilts selected from a background of limited feeding while the difference in yield of primal cuts between the two groups was not significant. Although the correlation between loin-eye area and primal-cut yield is high, the unexplained variation ($1-r^2$) remains high. The loin-eye area can be measured more accurately because it is much less affected by the errors inherent in the cutting procedure and in the variation of intermuscular fat. On this basis, the loin-eye area would represent a more accurate representation of total lean in the carcass than would the yield of trimmed primal cuts.

According to Cummings and Winters (1951), the length of side of the pork carcass is not significantly related to primal-cut yield. The findings of the present study agree with this previous work (table 3).

Hazel and Kline (1952) found a high correlation between carcass fatback and live backfat probes ($r=0.81$). In the present study, the gross and within-treatment group correlations between live backfat probes and carcass fatback measurements were highly significant (table 3). Thus, live backfat probes are a reliable measure of backfat thickness in live hogs.

Summary

Thirty-two gilts, from the eighth generation of an experiment designed to study the relationship between nutrition and improvement of animals for meat production through breeding were slaughtered to study carcass characteristics under four combinations of full and limited feeding.

The gilts selected from a background of full feeding showed more muscling than those selected from a background of limited feeding. The measure was the area of cross section of the longissimus dorsi muscle. Gilts from the background of full feeding averaged over ½ sq. in. greater development of loin eye ($P<0.01$). The results of this study indicate that a better job of selection for meat-type hogs, as based on muscular development, can be accomplished under full feeding, where the muscling is allowed to express itself to the fullest extent by selection time.

The plane of nutrition had a statistically significant effect upon carcass fatback ($P<0.01$). The full-fed gilts averaged 0.32 in. more backfat than gilts receiving 70% of full feed. When the 56.5 days difference in age between the two groups at slaughter was adjusted to a constant age, the full-fed gilts averaged 0.56 inch more backfat than the limited-fed gilts.

There was a statistically significant interaction between selection background and plane of nutrition on yield of trimmed primal cuts. Selection under full feeding favored a higher yield of trimmed primal cuts when such animals were placed on limited feeding. This genotype-environment interaction may partly determine whether breeding stock should be selected under the environment in which their progeny will be produced.

A highly significant correlation between live backfat probes and carcass fatback measurements was found.

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FAILURE TO DETECT RESIDUAL ESTROGENIC ACTIVITY IN THE EDIBLE TISSUES OF STEERS FED STILBESTROL

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BURROUGHS *et al.* (1954) reported growth stimulation of cattle which had been fed 2.5 to 10.0 mg. per head per day of the synthetic estrogen stilbestrol. Since then the practice of incorporating small amounts of stilbestrol in the feed of cattle being fattened for market has become widespread.

Because of the importance of beef in the diet, it is important to know whether estrogenic residues appear in the edible tissues of animals receiving the stilbestrol treatment. Perry *et al.* (1955), Turner (1956), and Preston *et al.* (1956) were unable to detect estrogenic activity in the tissues from steers which had been fed 10 mg. of stilbestrol per day. On the other hand, the estrogen assays of Stob *et al.* (1956) indicated estrogenic residues in tissues from steers fed stilbestrol at the rate of 10 mg. per day. Gossett *et al.* (1956) were able also to detect small amounts of estrogenic residues in the tissues from steers fed high levels of stilbestrol (up to 200 mg. per day).

During the summer of 1955, the U. S. Department of Agriculture at the Agricultural Research Center, Beltsville, Maryland, fed stilbestrol to steers in connection with a study on carcass quality and provided us with sufficient tissues for estrogen assays. Using an improved method of assay, we failed to detect estrogenic residues in the edible tissues of steers fed up to 60 mg. per day when the animals were taken off the stilbestrol feed 48 hours before slaughter.

Materials and Methods

The tissues for assay were obtained from 22 steers. Ten steers did not receive stilbestrol and were kept as controls. Ten steers received stilbestrol in a feed supplement at the rate of 10 mg. per steer per day. Five steers from each of these groups were slaughtered at the end of 90 days and the remainder were slaughtered at the end of a 180-day feeding period. To determine whether higher rates of feeding, which might inadvertently occur would result in detectable tissue residues, one additional steer was fed at the rate of 30 mg. per day and one was fed at the rate of 60 mg. per day for a 150-day feeding period. The animals were taken off the stilbestrol feed 48 hours before slaughter. At slaughter, tissues from each steer were collected and sharp frozen. The samples consisted of lean muscle, caul

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TABLE 1. MEAN UTERINE WEIGHT OF GROUPS OF SIX IMMATURE MICE FED STEER LEAN MUSCLE DIETS FOR SEVEN DAYS WITH VARIOUS LEVELS OF ADDED STILBESTROL

Assay number	Treated steer number	Mg. DES per day and number of days	Mean uterine weight in mg. at an added DES level of				Control steer number	Mean uterine weight in mg. at an added DES level of			
			0 ppb	2 ppb	4 ppb	6 ppb		0 ppb	2 ppb	4 ppb	6 ppb
1-a	209	10 mg.–90 days	10.1	11.9[a]	26.8[b]	63.7[c]	216	7.7	10.8[a]	44.5[b]	55.8[c]
1-b			7.2	9.7[a]	36.8[b]	63.9[c]		6.0	10.3[a]	37.5[b]	54.2[a]
2-a	210	10 mg.–90 days	6.5	17.0	29.8	43.6	214	5.6	10.7	20.8	31.1
2-b			8.8	9.8	12.9	30.3		7.0	9.3	18.0	29.1
3-a	208	10 mg.–90 days	7.4	11.1	19.0	35.8	203	7.0	9.4	19.2	20.9
3-b			6.8	12.3	18.7	31.4		7.6	10.9	17.5	29.0
4-a	189	10 mg.–90 days	6.7	9.3	16.6	45.1[d]	211	6.4	7.1	16.8	22.4[d]
4-b			6.0	9.6	22.0	46.6[d]		6.3	9.4	19.9	42.9[d]
5-a	201	10 mg.–90 days	6.4	8.7	25.0	37.0	190	7.4	9.1	19.1	29.2
5-b			6.2	9.6	15.8	31.0		...	10.1	15.1	26.8
6	337	30 mg.–150 days	11.8	14.4	21.4	36.9	190	9.8	12.2	20.7	36.1
7	343	60 mg.–150 days	7.9	10.8	28.0	34.4	190	9.4	10.1	22.3	35.5
8	185	10 mg.–180 days	6.7				194	6.7			
9	184	10 mg.–180 days	7.1				170	7.9			
	149		7.2				154	7.6			
	192		9.3				196	7.4			
	199		7.2				135	7.6			

In some assays, the amount of added stilbestrol differed from that shown; these were a=3 ppb; b=9 ppb; c=27 ppb; d=8 ppb.

fat, liver, kidney, and portions of the gastrointestinal tract. This latter sample of tripe and intestines was a composite of one-third large intestines, one-third small intestines, and one-third of a portion of one of the stomachs. They were washed thoroughly in running water.

The method of assay, the details of which are described elsewhere (Umberger *et al.*, 1958a), was based on the work of Stob *et al.* (1954), Umberger (1956), Turner (1956), and Preston *et al.* (1956). In brief, this method consisted of feeding the tissue diets to immature mice for

TABLE 2. MEAN UTERINE WEIGHT OF GROUPS OF SIX IMMATURE MICE FED VARIOUS STEER TISSUE DIETS FOR SEVEN DAYS WITHOUT ADDED STILBESTROL

	Composition of diet fed		Control		Treated	
Tissue	Parts tissue	Parts mouse diet	Number of assays	Average mean uterine weight	Number of assays	Average mean uterine weight
Lean	100	10	16	7.3 (5.6–9.8) [a]	17	7.6 (6.0–11.8)
Liver	100	10	12	8.3 (6.4–10.4)	12	8.9 (6.3–10.9)
Fat	100	200	11	20.0 (11.7–32.9)	13	17.1 (8.9–25.2)
Kidney	100	10	8	7.1 (6.2–7.8)	10	7.3 (6.4–8.0)
Tripe and intestine	100	30	11	6.9 (4.0–9.9)	12	6.8 (4.2–7.8)

[a] Values in parenthesis indicate range.

7 days, sacrificing, and weighing their uteri on the eighth day. The diets were prepared by grinding thawed tissue with laboratory mouse diet, making up in 6-gram patties, and refreezing.

With the exception of the fat diets, this amount of diet was all consumed and was nutritionally sufficient to produce some growth. Individual records of initial and final body weights and food consumption of each mouse were made.

In each assay, the mean uterine weight of mice fed tissues from a control steer was compared with the mean uterine weight of mice fed tissues from a treated steer. In general a two-curve design was used. This was accomplished by adding various concentrations of stilbestrol to each diet to establish a standard or control curve and an unknown or treated curve. For each assay 48 mice divided into groups of 6 were used.

Results and Discussion

To demonstrate the type of results obtained, a summary of the complete data obtained with steer lean muscle is given in table 1. In none of these assays was there statistically significant differences between the uterine weight response of mice fed control tissue and the uterine weight response of mice fed treated tissue. In order to determine what amount of estrogenic residues would have been detected if present, the sensitivity of the first 12 assays were calculated using the log of the ratio of uterine weight in milligrams to the final body weight in grams as the response. The mean sensitivity was 0.71±standard error 0.06 with a range of 0.22 to 1.05 parts per billion of stilbestrol.

Rather than present all of the data, a summary of the results of the assays on all tissues is given in table 2. It will be noted that no difference appears between the uterine weights of mice fed control tissue diets and those fed treated tissue diets. The uterine weight obtained appears to depend upon the type of diet fed—fat diets in particular giving a high uterine weight. The effect of dietary fat on the uterine weight response to estrogen is presented in another communication (Umberger *et al.*, 1958b).

Assays were run on tissues from individual steers rather than on combined lots of tissue from several steers to determine the magnitude of the variation of the uterotropic response of tissues from different steers. The response appeared to be quite uniform. Since we have shown that the naturally occurring estrogens estrone and estradiol-17β are 1/10 to 1/20 as active as stilbestrol in this assay method (Umberger *et al.*, 1958a), it is unlikely that any variation in the natural estrogen content of the tissues would have been detected.

Our results are in complete agreement with those of Perry *et al.* (1955), Turner (1956), Preston *et al.* (1956), Gossett *et al.* (1956), and Wiberg *et al.* (1957). The following comments are offered on the results of Stob *et al.* (1956) which indicated added estrogenic activity in lean muscle, kidney fat, liver, intestine, and kidney of steers fed stilbestrol at the rate of 10 mg. per day. As the hormone was fed up to the time of shipment for slaughter, it is not surprising that residual estrogen could be detected in liver, kidney, and intestine. It is unfortunate that the authors did not establish simultaneously a standard curve so that the amount of estrogen could be measured. The small differences noted in the uterine weights of mice fed control and treated tissues of muscle and kidney fat would probably have proved insignificant if the assay had been repeated using the design we have used here.

Mitchell (1957) fed tritium-labeled stilbestrol to a steer for 10 days at the rate of 10 mg. per day and slaughtered it 24 hours after the last feeding. On analyzing the tissues for residual radioactivity, he found residues equivalent to 0.30 parts per billion of stilbestrol in lean meat,

0.35 parts per billion in internal fat, 9.12 parts per billion in liver, and 4.15 parts per billion in kidney. None was detected in the heart tissue or in the blood. Mitchell did not identify the compound or compounds containing the residual radioactivity, nor did he test the residues for estrogenic activity by bioassay. It is interesting, however, that Gossett *et al.* (1956) under the same conditions (i.e., feeding 10 mg. per day until 24 hours before slaughter) found 6 parts per billion stilbestrol equivalents in liver by using the mouse uterine weight method as compared to 9 parts per billion stilbestrol equivalents found by Mitchell in using tritium-labeled stilbestrol. These values are quite close when one considers the difference in the two methods. However, Gossett *et al.* (1956) failed to detect activity in the kidney.

Summary

No added estrogenic activity could be detected in the edible tissues of steers fed 10, 30, and 60 mg. of stilbestrol per steer per day for 90 to 180 days when the stilbestrol was removed from the feed 48 hours before slaughter. The method used was capable of detecting 2 parts per billion in stilbestrol equivalents in every case, and often as low as 0.5 parts per billion. The failure to detect estrogenic residues in the edible tissues of one steer fed at the rate of 30 mg. per day and another at the rate of 60 mg. per day, when the feed was removed 48 hours before slaughter, provides a safety factor for the possible misuse of the products.

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THE EFFECT OF DIETHYLSTILBESTROL, RESERPINE, HYDROXYZINE AND HYGROMYCIN ON GROWTH AND CARCASS QUALITY OF LAMBS [1, 2, 3]

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NUMEROUS reports of the growth stimulatory effects of diethylstilbestrol (DES) administered either by subcutaneous implantation or *per os* in the ration of lambs have been published (Andrews *et al.*, 1949, 1956; Jordan, 1950; Jordan *et al.*, 1956; Means *et al.*, 1953; O'Mary *et al.*, 1952; Taylor *et al.*, 1957). In several instances a marked lowering of carcass quality of lambs treated with DES has been observed. There is current interest in compounds which have a tranquilizing effect in man and laboratory animals.

Andrews *et al.* (1958) observed that while there was an apparent growth depression in lambs which received 500 mcg. of reserpine per pound of ration there was an indication that carcass quality was improved.

The experiment reported in this paper was conducted to determine the effect on growth, feed consumption, feed efficiency, and carcass quality of (1) two different tranquilizers (reserpine and hydroxyzine) when each is fed at two different levels, (2) an anthelmintic, hygromycin, and (3) implanted DES superimposed on each of the above treatments.

Materials and Methods

Two-hundred and forty western ewe and wether lambs, averaging 75 lb. liveweight, were divided into 12 lots of 20 lambs each to be fed a pelleted, complete mixed fattening ration. The percentage composition of the control ration was as follows: Ground corn cobs, 39.5; dehydrated alfalfa meal, 20.0; ground yellow corn, 32.0; soybean oil meal, 7.0; salt (with cobalt), 0.5; steamed bonemeal, 1.0; vitamin A and D concentrate, 0.075. The salt contained one ounce cobalt carbonate per 100 lb.; 0.075% vitamin concentrate (1.5 pound per ton) gave 3333 USP units of vitamin A and 425 USP units of vitamin D per pound.

A 6 x 2 factorial feeding trial in which diethylstilbestrol implantation was superimposed upon the second replication of a control and five treatments was used. The five treatments, in addition to the control, consisted of: (a) 5 mcg. reserpine, (b) 10 mcg. reserpine, (c) 1 mg. hydroxyzine, (d) 2 mg. hydroxyzine, and 3.5 mg. hygromycin, per pound of total ration, respec-

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[2] Diethylstilbestrol, reserpine and hydroxyzine were furnished by Charles Pfizer and Company. Hygromycin was furnished by Eli Lilly and Company.

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TABLE 1. THE EFFECT OF DIETHYLSTILBESTROL, RESERPINE (RES.), HYDROXYZINE (HYDROX.), AND HYGROMYCIN (HYGRO.), ON GROWTH AND CARCASS QUALITY OF LAMBS NOVEMBER 8, 1957–JANUARY 7, 1958 (60 DAYS) [a]

	No diethylstilbestrol						3 mg. diethylstilbestrol implant					
Item	I Control	II 5 mcg. Res.	III 10 mcg. Res.	IV 1 mg. Hydrox.	V 2 mg. Hydrox.	VI 3.5 mg. Hygro.	VII Control	VIII 5 mcg. Res.	IX 10 mcg. Res.	X 1 mg. Hydrox.	XI 2 mg. Hydrox.	XII 3.5 mg. Hygro.
No. of lambs	20	20	20	20	20	20	20	20	20	20	20	20
Av. initial wt., lb.	75	75	75	74	75	75	74	75	75	75	75	75
Av. final wt., lb.	107	108	108	107	107	105	116	114	117	115	117	112
Total gain, lb.[b]	32	33	33	33	32	30	42	39	42	40	42	37
Av. daily gain, lb.	0.54	0.56	0.56	0.55	0.53	0.51	0.70	0.66	0.70	0.67	0.71	0.62
Daily feed per lamb pelleted ration, lb.	4.6	4.6	4.8	4.6	4.6	4.2	4.7	4.8	4.8	4.8	4.9	4.4
Feed per lb. gain, pelleted ration, lb.	8.5	8.3	8.5	8.5	8.7	8.4	6.7	7.3	6.8	7.1	6.9	7.0
Federal carcass grades												
No. high choice	0	1	0	0	0	0	0	0	0	0	0	0
No. average choice	3	6	4	3	2	3	1	1	2	4	2	0
No. low choice	9	8	12	8	10	2	4	6	7	5	3	4
No. choice	12	15	16	11	12	5	5	7	9	9	5	4
% choice	60	75	80	55	60	25	25	35	45	45	25	20
No. high good	3	4	1	6	5	7	3	5	3	5	7	4
No. average good	5	1	3	3	3	5	9	6	5	4	5	7
No. low good	0	0	0	0	0	3	3	2	3	2	3	2
No. good	8	5	4	9	8	15	15	13	11	11	15	16
% good	40	25	20	45	40	75	75	65	55	55	75	80
Average numerical grade[c]	6.50	7.10	6.85	6.55	6.55	5.85	5.55	5.90	6.00	6.25	5.80	5.35

[a] Amounts of reserpine, hydroxyzine and hygromycin are per pound of total ration.

[b] LSD for total gain, 5%, 3.31 lb.; 1%, 4.37 lb.

[c] Numerical designation of grades were as follows: High choice 9; average choice 8; low choice 7; high good 6; average good 5; low good 4. LSD for numerical grade, 5%, 0.69; 1%, 0.91.

tively. At the conclusion of the trial all lambs were slaughtered and U.S. carcass grade was assigned to each carcass by a government grader.

Results and Discussion

Two tranquilizers, reserpine and hydroxyzine, when each was fed at two levels, exerted no significant effect on the growth rate of fattening feeder lambs fed a pelleted complete ration (table 1). However, it should be noted that both the negative and positive control groups (Lots I and VII) gained exceedingly well (0.54 and 0.70 lb. per day, respectively). Andrews et al. (1958) reported no significant increase in growth rate from feeding 0.25 mg. reserpine per lb. of ration, whereas a level of 0.50 mg. per lb. of ration appeared to depress the growth rate of fattening lambs. However, the levels of reserpine fed in the previous trials at this station (Andrews, *et al.*, 1958) were from 25 to 50 times as great as those fed in the trial reported in this paper (5 mcg. and 10 mcg.) Apparently the range of levels at which reserpine may be fed to lambs with no observable detrimental effect is quite large. The research of Sherman *et al.* (1957) indicated a 14% increase in gain from the feeding of a ration containing 0.6 mg. hydroxyzine, and a 28% increase in gain from a ration containing 1 mg. hydroxyzine per lb. of ration when fed in a pelleted complete ration similar to that fed in the Purdue trial.

The inclusion of hygromycin in the ration had no significant effect on the gains of lambs which were not implanted with diethylstilbestrol (Lot VI), but did result in a significant depression ($P<0.05$) in the gain of lambs that had been implanted with 3 mg. diethylstilbestrol. It should be pointed out that all lambs were wormed with a standard nicotine sulfate-copper sulfate drench at the start of the trial. This may have affected the response obtained with the hygromycin. Stroud *et al.* (1957) reported a significant increase in the rate of gain ($P<0.05$) from the feeding of 5 mg. hygromycin A per lb. total ration.

The implantation of 3 mg. diethylstilbestrol per lamb (Lots VII-XII) resulted in highly significantly increased rates of gain ($P<0.01$) over the lambs not implanted (Lots I-VI). This is in accord with the results of several similar trials. For comparative purposes, numerical values were assigned to the carcass grades as shown in table 1. An analysis of variance of the numerical scores indicates that the implantation of diethylstilbestrol resulted in a lowering of the quality of carcasses produced. This too is in accord with the results reported by Andrews *et al.* (1958, 1956), Jordan (1950) Jordan *et al.* (1956) and others. In work reported from this Station, government graders have down-graded carcasses from lambs administered diethylstilbestrol because of less fat-covering on the outside, less "feathering" on the ribs, and because such carcasses did not firm as rapidly or completely as the carcasses from lambs which had received no diethylstilbestrol.

The castrated males gained more than the females under the conditions of this trial (table 2). This difference which was statistically highly significant occurred in the presence of, as well as in the absence of, diethylstilbestrol implants, tranquilizers and hygromycin.

Summary

Two levels of reserpine (5 mcg. and 10 mcg.) and two levels of hydroxyzine (1 mg. and 2 mg.) per lb. of pelleted complete ration had no significant effect on the rate of gain of fattening lambs in drylot. However, the control lambs which received no reserpine or hydroxyzine gained exceedingly well (control not implanted with diethylstilbestrol, 0.54 lb. per day; control implanted with diethylstilbestrol, 0.70 lb. per day).

TABLE 2. EFFECT OF SEX ON RATE OF GAIN OF FEEDER LAMBS

	Lots I to VI		Lots VII to XII	
	No diethylstilbestrol		3 mg. diethylstilbestrol implant	
Item	Castrate male	Female	Castrate male	Female
Number	49	71	53	67
Initial wt., lb.	75.3	74.1	75.2	74.6
Final wt., lb.	110.6	104.7	117.8	113.4
Total gain, lb.	35.2	30.6	42.6	38.9

Lambs implanted with 3 mg. diethylstilbestrol gained significantly more than lambs not implanted. The carcasses from the implanted lambs were graded lower than those from the non-implanted lambs.

The inclusion of hygromycin at a level of 3.5 mg. hygromycin per lb. total ration had no significant effect on the gains of lambs which were not implanted with diethylstilbestrol, but did result in a significant depression in the gain of lambs that had been implanted with 3 mg. diethylstilbestrol.

Castrate males gained more than females. This consistent difference occurred in the presence of, as well as in the absence of, diethylstilbestrol implants, tranquilizers and hygromycin.

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SENSITIVITY OF VARIOUS TISSUES OF HOLSTEIN CALVES TO TOCOPHEROL INTAKE [1]

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SINCE the classical studies of Evans and Burr (1927), it has been recognized that tocopherol, as determined by rat assay procedures, is widely distributed throughout the animal body. More recently, these observations have been confirmed by Mason (1942) with rats, Bratzler *et al.* (1950) with swine, Blaxter *et al.* (1952) with calves, and Mecchi *et al.* (1956) with poultry. The feasibility of using the tocopherol concentration in tissues to estimate the relative potency of tocopherol from various dietary sources has also been recently demonstrated in several different species (Bolliger and Bolliger-Quaife, 1955; Bunnell, 1957; Eaton *et al.*, 1958). The present study was concerned with determining the relative sensitivity in the dairy calf, of liver, heart, trapezius muscle, and perinephric fat tocopherol concentrations to graded levels of tocopherol intake.

Experimental Procedure

Animals and Rations. Calf tissues from three experiments conducted at the Storrs (Conn.) Station were used in this comparative study. In the first (I) experiment, 40 one-day old Holstein male calves, obtained from the University of Connecticut and other state institution herds, were raised to 63 days of age on a standard regime of limited whole milk, limited starter and *ad libitum* U. S. no. 2 mixed hay. On the 64th day of age, each calf was fed a basal ration, containing relatively low amounts of carotene and tocopherol, at a daily rate to provide an anticipated 7-day increase in live weight of 10 lb. In addition, vitamin A was fed at the daily rate of 40 mcg. per lb. of live weight. On the 92nd day of age, each calf was fed in addition to the basal ration, one of five levels

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of tocopherol from a dry carrier, 0.000, 0.128, 0.256, 0.512, or 1.024 mg. of free tocopherol per lb. live weight per day, or one of five levels of tocopherol from artificially dehydrated alfalfa leaf meal, 0.000, 0.125, 0.250, 0.500 or 1.000 mg. The vitamin A intake was increased at this age to a 60 mcg. level in those calves receiving the tocopherol from the dry carrier. Those calves receiving tocopherol from the artificially dehydrated alfalfa received an amount of vitamin A equal to the difference of the pro-vitamin, carotene, in the alfalfa fed and the 60 mcg. vitamin A intake level. The vitamin A equivalencies of the carotene were calculated from the date of Rousseau *et al.* (1956). Each calf was slaughtered upon completion of the 12-week tocopherol supplementation period.

In the second (II) experiment, nine Holstein calves were raised to an average of 60 days of age (range 35 to 63) on a standard regimen of limited whole milk, limited starter and *ad libitum* U. S. no. 2 mixed hay. At an average age of 96 days (range 56 to 105) all calves were fed the same basal ration at the same level of intake as in experiment I. Vitamin A from a dry carrier was added at the rate of 6 mcg. per lb. of live weight per day. At an average age of 97 days each calf was fed, in addition to the basal ration, one of three levels of tocopherol from a dry carrier 1, 5 or 25 mg. of free tocopherol per lb. of live weight per day and one of three levels of vitamin A from a dry carrier 10, 100, 1000 mcg. per lb. of live weight per day. Each calf was slaughtered upon completion of the 4-week, tocopherol-vitamin A supplementation period.

In the third (III) experiment, 36 calves were raised to 35 days of age on a standard regimen of limited whole milk, limited starter and *ad libitum* U.S. no. 2 mixed hay. Each calf was then fed the same basal ration and at the same intake level as in experiments I and II, and vitamin A at the 6 mcg. intake level until each calf was 63 days of age. On the 64th day of age each calf was fed in addition to the basal ration one of three levels of tocopherol from a dry carrier, 1, 5, or 25 mg. of free tocopherol per lb. of live weight per day and one of three levels of vitamin A from a dry carrier, 10, 100, 1000 mcg. per lb. of live weight per day. Eighteen of the calves were slaughtered upon the completion of the 4-week, tocopherol-vitamin A supplementation period and 18 were fed the basal ration without tocopherol or vitamin A supplements until the blood plasma vitamin A level decreased to less than 4.0 mcg. per 100 ml. for two consecutive 7-day periods. For purposes of this study, only the data from the 18 calves which were slaughtered after the tocopherol-vitamin A supplementation period were used.

More detailed information can be found for experiment I by reference to Eaton *et al.* (1958) and for experiment III by reference to Dicks *et al.* (1958). Experiment II represents unpublished data of a pilot study (Eaton *et al.*, 1957).

Observations and Analyses. In all experiments, feeds fed and refused, except the vitamin supplements, were weighed to the nearest 0.1 lb. The

artificially dehydrated alfalfa leaf meal was weighed to the nearest 0.1 gm. and the dry carriers of tocopherol and vitamin A were weighed to the nearest 0.01 gm. To insure complete consumption, these were mixed and fed in a small portion of feed prior to feeding the balance of the ration allowance. Primarily for the purpose of adjusting the vitamin intakes to live weights at successive 7-day intervals, live weights were recorded to the nearest pound at the start of the experiment and thereafter at successive 7-day intervals.

At the termination of each animal's vitamin supplementation period, the animal was slaughtered and samples of the heart, liver, trapezius muscle and perinephric fat were obtained and stored at 0° F. for subsequent analysis.

Total tocopherols and lipids in the tissues were analyzed in duplicate by a modification (Bunnell, 1955) of the method of Quaife and Harris (1948). Chromatography of the molecular distillates on Floridin (Florex, XXS, 60–90 mesh, Floridin Co., Tallahassee, Fla.) was employed to remove carotenoids (Brown, 1952). Perinephric fat samples, however, were saponified (Eggitt and Ward 1953) prior to molecular distillation and chromatography. Dry matter was determined in duplicate by drying samples in an oven for 24 hours at 100° C.

Statistical procedures as outlined by Cochran and Cox (1957) and Snedecor (1956) were used in the analysis of the data.

Results and Discussion

In table 1 the following data are presented by experiments: the mean log tocopherol concentrations of liver, heart, trapezius muscle and perinephric fat on a wet basis and on a lipid basis, the standard deviation per animal for these criteria, and the standard deviations as a percentage of their respective means, the coefficients of variation. Since it was observed that in all experiments an increase in variability of tissue tocopherol concentration occurred with an increase in intake (the standard deviations were approximately proportional to the mean concentration from which the coefficient of variation was derived), these response criteria were transformed to their respective logarithms (base 10) to minimize differences in variation of the tissue tocopherol concentrations, and thereby meet the assumption of constant variability for analysis of variance procedures.

Across all experiments, the average of the mean log concentrations of mcg. tocopherol per 100 gm. of tissue, weighted for the number of animals in each experiment, were: Perinephric fat 3.28, heart 2.77, liver 2.70 and trapezius muscle 2.49 expressed on a wet basis. When tocopherol concentration was expressed on a lipid basis as log of the mcg. of tocopherol per gram of lipid, the following results were obtained: Trapezius muscle 2.41, heart 2.32, liver 2.12 and perinephric fat 1.37. By expressing the mean log

tocopherol concentration on a lipid basis, the order of magnitude of concentration of the various tissues were almost completely reversed to that obtained on a wet basis. This is due, largely, to the lipid concentrations, which were 81.5 gm. % for perinephric fat, 3.8 for liver, 2.7 for heart and

TABLE 1. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF LIVER, HEART, TRAPEZIUS MUSCLE AND PERINEPHRIC FAT TOCOPHEROL CONCENTRATIONS

	Experiments		
	I	II	III
Tocopherol intake (mg./lb. live wt./d.)	0.00–1.02	1.00–25.00	1.00–25.00
Duration of supplement feeding (wk.)	12	4	4
Total no. of calves	40	9	18
No. of calves per tocopherol intake group	4	3	6
Error degrees of freedom	30	3	9
Tocopherol concentration—wet basis		log (mcg./100 g.)	
Liver			
Mean ($\bar{y}$)	2.30	3.24	3.31
Standard deviation (s_y)	0.11	0.14	0.07
Coefficient of variation (C.V.)	4.8	4.3	2.1
Heart			
Mean ($\bar{y}$)	2.54	3.14	3.09
Standard deviation (s_y)	0.10	0.07	0.06
Coefficient of variation (C.V.)	3.9	2.2	1.9
Trapezius muscle			
Mean ($\bar{y}$)	2.37	2.73	2.64
Standard deviation (s_y)	0.10	0.03	0.07
Coefficient of variation (C.V.)	4.2	1.1	2.6
Perinephric fat			
Mean ($\bar{y}$)	3.15	3.52	3.46
Standard deviation (s_y)	0.10	0.07	0.11
Coefficient of variation (C.V.)	3.2	2.0	3.2
Tocopherol concentration—lipid basis		log [(mcg./100 g.)/(g. lipid/100 g.)]	
Liver			
Mean ($\bar{y}$)	1.73	2.64	2.71
Standard deviation (s_y)	0.12	0.13	0.06
Coefficient of variation (C.V.)	6.9	4.9	2.2
Heart			
Mean ($\bar{y}$)	2.07	2.68	2.70
Standard deviation (s_y)	0.11	0.04	0.07
Coefficient of variation (C.V.)	5.3	1.5	2.6
Trapezius muscle			
Mean ($\bar{y}$)	2.28	2.66	2.59
Standard deviation (s_y)	0.09	0.04	0.06
Coefficient of variation (C.V.)	3.9	1.5	2.3
Perinephric fat			
Mean ($\bar{y}$)	1.23	1.61	1.57
Standard deviation (s_y)	0.10	0.08	0.12
Coefficient of variation (C.V.)	8.1	5.0	7.6

1.2 for trapezius muscle, all values representing means weighted for the number of animals in each experiment. Average coefficients of variation, weighted for the error degrees of freedom in each experiment, were on a wet basis as follows: Liver 4.2%, trapezius muscle 3.6%, heart 3.4% and perinephric fat 3.1% and on a lipid basis: perinephric fat 7.8%, liver 5.8%, heart 4.4% and trapezius muscle 3.4%. Similar to the mean log tocopherol concentration, the order of magnitude of the coefficients of variation was changed when the concentration of tocopherol was expressed on a lipid basis. In general, the coefficients of variation of log tocopherol concentrations per gram of lipid were slightly higher. This was possibly due in part to the errors involved in two analytical determinations in the calculation of the concentration of tocopherol on a lipid basis, one being the lipid determination and the other being the determination of the tocopherol, while, in computing the tocopherol on a wet tissue basis, only the errors inherent in the determination of tocopherol concentration were involved.

Decisions as to the choice of a criterion to measure response to a particular treatment are generally based on considerations of the magnitude of response as well as some estimate of experimental error so that the ratio of the response to the error becomes maximum. In this study, the sensitivity concept of Mandel and Stiehler (1954) was used to determine the relative merit of the various tissues for reflecting tocopherol intake. This concept defines sensitivity as the ratio of the rate of change of a criterion with respect to treatment, dy/dx, to the standard deviation per calf of the criterion, s_y. When the response is essentially linear, the ratio of the linear rate of change, b, to s_y is used (i.e., in this study, the rate of change of the logarithmic tissue tocopherol concentration per logarithmic unit of tocopherol intake to the standard deviation of the logarithmic tissue tocopherol concentration.) The rate of change is divided by the standard deviation in order to obtain a value independent of the unit used in measurement of the response criterion. Thus, tests of significance can be made to determine which of two criteria is more sensitive with the restriction that treatment (tocopherol intake in this study) be expressed in the same scale and within the same limits for both criteria. Advantages of this ratio are, that it remains constant for all values of treatment in which the response of a criterion is linear, that unlike the standard deviation and coefficient of variation, the ratio is invariant with respect to any transformation of the criterion.

For purposes of determining sensitivity, linear regressions of log tocopherol tissue concentration on log tocopherol intake and of log tocopherol tissue concentration per gram of lipid on log tocopherol intake were derived and are presented in table 2. This choice of expressing the functional relationship between tissue concentration and intake of tocopherol was based on a previous study (Rousseau *et al.*, 1957) in which constant increases in liver concentration on intake (i.e. levels of intake added to a

basal ration) were found when both concentrations and intake were transformed to logarithms (base 10). In the case of experiment I, the groups of calves receiving no added tocopherol to the basal ration were not used in the calculations of the regressions. These calves were omitted since use of the logarithmic transformation of both the response and intake variables resulted in curvature. The curvature was apparently due to the mean tissue concentrations of the no added tocopherol group being not equal to zero as

TABLE 2. SENSITIVITY OF VARIOUS TISSUES TO TOCOPHEROL INTAKE

Tissue and experiment	Linear slope of log tissue concentration on log intake of tocopherol		Sensitivity	
	Wet basis	Lipid basis	Wet basis	Lipid basis
	b		S[a]	
Liver				
I Dry carrier	0.50	0.49	4.5	4.1
Alfalfa	0.33	0.34	3.0	2.8
II Dry carrier	0.58	0.59	4.1	4.5
III Dry carrier	0.54	0.53	7.7	8.8
Heart				
I Dry carrier	0.58	0.64	5.8	5.8
Alfalfa	0.36	0.36	3.6	3.3
II Dry carrier	0.27	0.28	3.8	7.0
III Dry carrier	0.35	0.36	5.8	5.1
Trapezius muscle				
I Dry carrier	0.28	0.32	2.8	3.6
Alfalfa	0.12	0.22	1.2	2.4
II Dry carrier	0.13	0.12	4.3	3.0
III Dry carrer	0.29	0.30	4.1	5.0
Perinephric fat				
I Dry carrier	0.30	0.31	3.0	3.1
Alfalfa	0.19	0.21	1.9	2.1
II Dry carrier	0.20	0.20	2.8	2.5
III Dry carrier	0.30	0.30	2.7	2.5

[a] b/s_y

discussed by Ames and Harris (1956). Correlation coefficients between intake and mean response ranged between 0.89 and 1.00 when log tissue concentration was expressed on a wet basis and between 0.85 and 1.00 when expressed on a lipid basis, indicating essentially constant relationships between log tissue concentration and log intake. Thus the assumption of linear rates of change required in the use of b/s_y in the derivation of sensitivity was realized. Average sensitivities, weighted for the error degrees of freedom in each experiment, were on a wet basis: Heart 4.8, liver 4.2, perinephric fat 2.5, trapezius muscle 2.4, and on a lipid basis: Heart 4.7, liver 4.2, trapezius muscle 3.2 and perinephric fat 2.6.

In general, heart was the tissue found to be the most sensitive to

tocopherol intake, followed closely by liver, while perinephric fat and trapezius muscle were somewhat lower than the preceding two tissues. Tests of significance among individual tissue sensitivities by experiments at the 95% probability level, as outlined by Mandel and Stiehler (1954) indicated, in general, that there were no statistically significant differences in sensitivity between liver and heart or between trapezius muscle and perinephric fat. However, the first mentioned pair of tissues were of significantly greater sensitivity in reflecting tocopherol intake than trapezius muscle or perinephric fat. These above mentioned differences between tissues existed whether tocopherol concentration was expressed on a wet or on a lipid basis.

The general application of these findings, that heart or liver are more sensitive to tocopherol intake than trapezius muscle or perinephric fat, is that to obtain a specified statistically significant difference between two tocopherol intakes, fewer calves per intake level are needed in the case of the first named pair of tissues than in the case of the second pair. For purposes of illustrating this, the number of replications required to determine the difference (5% level and 80% probability; one tailed test, since it is reasonable to assume that tocopherol added to the ration will not result in decreased tissue concentration; and three intake levels or treatments—see Section 2.21 of Cochran and Cox, 1957) in logarithmic tissue concentration due to increasing the intake by one-half of a logarithmic unit from the dry carrier were calculated. Based on the percentage ratio of one-half of the average linear slopes (weighted for the number of animals in each experiment fed this source of tocopherol) to their respective weighted average logarithmic tissue concentration (liver 9.63%, heart 7.94%, trapezius muscle 5.22% and perinephric fat 4.27%) and the weighted average coefficients of variation (see above), four replications were required in the case of liver, three in the case of heart, eight in the case of trapezius muscle, and nine in the case of perinephric fat.

It would appear from these studies that either heart or liver are the tissues of choice for the purpose of reflecting tocopherol intake of the dairy calf. In the case of investigations dealing with both tocopherol and vitamin A, the liver would be preferred, for it is the major storage site of vitamin A as contrasted to minimal storage of vitamin A in the heart. Slight changes in sensitivity were found to occur when tocopherol concentration was expressed as per gram of lipid as compared to concentration on a wet basis. Therefore, it would seem that little is to be gained with respect to increasing sensitivity by the conversion of tocopherol concentration from a wet basis to a lipid basis. Whether these general findings apply to other experimental conditions, such as when cod liver oil or other sources of unsaturated fat are fed, awaits collection and quantitative analyses of such data.

Summary

Data obtained at the termination of three experiments on the total tocopherol concentrations of liver, heart, trapezius muscle and perinephric fat of Holstein male calves, receiving graded levels of tocopherol added to a basal ration in daily amounts ranging from 0.125 to 25.000 mg. per lb. of live weight and for periods ranging from 4 to 12 weeks duration, were studied for the purpose of determining the relative sensitivity of these tissues in reflecting tocopherol intake. Using the sensitivity concept of Mandel and Stiehler (1954), which in this study was the ratio of the rate of linear change of the logarithmic (base 10) tissue concentration of tocopherol on the logarithmic intake of tocopherol to the standard deviation per calf of the logarithmic tissue tocopherol concentration, it was found that the sensitivity was in the following descending order: heart, liver, perinephric fat and trapezius muscle. The first two named tissues were found to be approximately twice as sensitive to tocopherol intake as the latter two. Expressing tocopherol concentration on a per gram of lipid basis resulted in inappreciable change in sensitivity as compared to a wet basis as used above.

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EFFECT OF SEVERAL COMBINATIONS OF GRASS AND GRAIN IN THE RATION OF BEEF STEERS ON THE COLOR CHARACTERISTICS OF LEAN AND FAT [1]

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THE color of lean and in some cases the color of fat are two of the criteria by which consumers measure the quality and desirability of beef. The kind of feed or more specifically the use of grass alone or grass in combination with grain has been implicated as one of several factors contributing to the color of lean (Black *et al.*, 1940; Bull *et al.*, 1941). Some packers claim that ribbed carcasses and other fresh cut surfaces from pasture-fed cattle do not retain their bright color as well as those coming from grain-fed cattle. It is also claimed that there is more moisture loss from grass-fed cattle and that this loss is manifested by a concentration of moisture on the cut surface of the ribeye. Yellow fat in beef has also been associated with the type of feed consumed and here again pasture has been shown to be one of the feeds involved (Wanderstock and Miller, 1948; Black *et al.*, 1940 Bull *et al.*, 1941).

In view of the discrimination against beef obtained from cattle fattened on grass, it was thought pertinent to study the effects of pasture feeding regimen on the color of external fat and the stability of color of lean and the moisture losses during cooler storage.

Materials and Methods

Sixty grade Hereford yearling steers of known feeding, breeding and management history were randomly allotted to six treatments of 10 steers each and fed the following rations for a period of approximately 6 months: treatment 1, pasture only; treatment 2, pasture plus ground corn (No. 2 yellow) amounting to 0.5% body weight daily; treatment 3, pasture plus a grain supplement amounting to 1.0% body weight daily (10% animal fat added to the corn); treatment 4, same as treatment 3 except that no animal fat was added; treatment 5, pasture as the only source of feed for 3 months and ground corn (1.5% body weight daily) in addition to pasture for the last 3 months; treatment 6, a dry lot ration composed of ground ear corn and supplement with some added ground corn cobs.

At the time of slaughter most of the carcasses graded good and low choice. The carcasses were chilled at 35° F. for 48 hours and the right rib removed for chemical and physical determinations. The *longissimus dorsi* muscle

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(eye muscle) from the standard 9–10–11th rib section was removed for use in the various color measurements. A sample one-half inch thick was taken from the area of the 11th rib and the color immediately read on a Hunter Color and Color Difference Meter which had been standardized with a Gardner Laboratory red plate No. A-78. A second reading of the same surface was taken after a period of 30 min. in order to allow time for oxygenation of the cut surface. The brightening of the surface in cut beef muscle takes place very rapidly during the first 30 min. after cutting (Mackintosh and Hall, 1935).

The blade end of the 6–7–8th rib section was immersed in rendered beef fat and stored in a lighted cooler for 7 days at 34° F. and a relative humidity of 88%. This process sealed the blade end of the rib and exposed the cut surface of the 8th rib area to the cooler atmosphere. This procedure was undertaken to study the moisture concentration or migration that some packers claim they have encountered as well as the effects of cooler atmosphere on the cut surface of the rib. After aging, the color of the eye muscle from the 6–7–8th rib section was determined with the Hunter meter.

Total pigment. Since myoglobin, hemoglobin (as residual blood) and other heme pigments contribute to the color of beef, the "total" pigment was extracted from the ribeye muscle. The sample used for this purpose was taken adjacent to the one mentioned above and consisted of a slice one-half inch thick. It was trimmed free of external fat and connective tissue and ground three times in a hand food chopper to insure homogeneity. Duplicate aliquots of 25 gm. each were taken and the procedure used was the one described by Husaini *et al.* (1950) with the following modifications: After shaking, the meat-water mixture on a mechanical shaker for 10 min., it was centrifuged for 15 min. at 3500 RPM to remove as many of the tissue particles as possible; some additional material (mostly fat droplets) was removed by filtering through a small, loosely packed cotton wad and the solution was then filtered through Whatman No. 3 filter paper.

Myoglobin. In order to determine myoglobin concentration, a sample adjacent to the one for total pigment was used. Duplicate 25-gram samples were taken from the slice which had been prepared as mentioned above and the method outlined by Ginger *et al.* (1954) was used with slight modifications: The sample was blended in a Waring blendor with 125 ml. of cold distilled water for three minutes; the solution was transferred to a 250 ml. polyethylene bottle, 0.15 gm. of sodium carbonate added and the bottle shaken on a mechanical shaker for 10 min.; the samples were centrifuged for 15 min. at 3500 RPM; after addition of lead acetate and additional centrifugation the pH of this solution was adjusted to 6.6 prior to addition of the phosphates; a third centrifugation greatly aided in filtering the solution through Whatman No. 3 filter paper.

The calculations of the amount of myoglobin in milligrams per gram of fresh tissue was accomplished as follows: The molecular extinction coeffi-

cient is assumed to be 11,500, the same as that given by Drabkin and Austin (1935); using the value 16,500 for the molecular weight of myoglobin and allowing for dilution, the following expression was set up:

$$\frac{16{,}500}{11{,}500} \times \frac{150}{25} \times \frac{125}{100} \times \frac{100}{98} = 10.98$$

This constant times the optical density of the cyanmetmyoglobin solution gives the concentration of myoglobin in milligrams per gram of fresh tissue. The 100/98 relationship came about because 2 ml. of the solution was removed and replaced with 2 ml. of a solution of potassium ferricyanide and sodium cyanide.

Color of Fat. The fat samples for this study were taken from the exterior of the rib after which they were frozen and stored at 0° F. until used. They were then thawed, ground, melted in an oven and strained through cheese cloth into test tubes. Carbon dioxide was bubbled through the melted fat and the samples stoppered, sealed with paraffin and refrigerated until ready for use.

The tubes of fat were melted in an oven at low temperatures and a 7.5-gm. sample of melted fat was made to a volume of 25 ml. with hexane, shaken and read on a Beckman DU spectrophotometer. The samples were read at wave length 452 mu (Gortner *et al.*, 1949) after a study of several of them indicated that this was the point of maximum absorption.

Results and Discussion

Table 1 provides a treatment comparison between the lean color values obtained 30 min. after cutting with those obtained after aging. As a result of aging, lower values were found for all samples in brightness, redness and yellowness as determined with the Hunter meter on the *Rd*, *a* and *b* scales, respectively. This reduction in color value and the narrower color range found between treatments were probably due to the masking effect obtained as a result of the dried and darkened sample surface. A significant difference at the 0.05 level was found between treatments 4 and 6, read 30 min. after cutting. However, after aging, the *Rd* values for both of these treatment means were higher than those obtained for the others. These results may be explained on the basis of these two treatments producing the most fat (table 3) in the meat, and therefore it is possible that more moisture was retained resulting in a brighter color. Another possible explanation is that the samples with the greatest amount of fat reflected the most light. It would thus appear that any differences during cooler storage in color stability of lean was due to the amount of fat present rather than to the influence of treatments.

In order that the color difference between treatments could be studied at a point slightly below the exposed aged surface, a slice one-half inch thick was removed from the top of each sample and the freshly cut surface

TABLE 1. HUNTER COLOR AND COLOR DIFFERENCE VALUES OBTAINED BEFORE AND AFTER AGING BEEF RIBEYE MUSCLE [a]

Treatment [b]	Initial [c]			After 30 minutes			Aged surface after 7 days			½" below aged surface		
	Bright-ness	Red-ness	Yellow-ness	Bright-ness	Red-ness	Yellow-ness	Bright-ness	Red-ness	Yellow-ness	Bright-ness	Red-ness	Yellow-ness
1	8.3	32.9	13.1	8.3	35.2	14.8	6.0	33.8	12.1	7.8	34.6	12.7
2	7.4	35.5	13.1	7.7	39.3	15.2	6.2	36.2	11.8	7.8	35.3	12.2
3	8.2	34.5	14.0	8.0	38.4	15.6	5.6	36.3	12.2	7.6	36.8	13.6
4	7.7	37.1	14.4	7.4	41.2	15.6	6.4	36.8	12.7	7.7	36.1	13.6
5	8.3	33.9	13.6	8.0	37.5	15.1	6.0	33.9	11.0	7.5	36.1	12.3
6	9.3	35.3	14.9	9.4	36.8	15.5	7.0	34.6	11.9	9.8	34.2	13.2

[a] Twenty units were arbitrarily added to each redness value in order that conversion could be made to Munsell values.
[b] Mean of 10 animals. Description of treatments may be found in Materials and Methods section
[c] The brightness, redness and yellowness values were determined with the Hunter instrument *Rd*, *a* and *b* scales, respectively. The higher the numerical value, the more pronounced is the color characteristic.

immediately read on the Hunter meter. Only treatment 6 was different in brightness from the other treatments at the 0.05 level of significance. Thus, the meat coming from the dry-lot cattle was brighter under all four conditions of measurement (table 1). It should be noted, however, that the *Rd* values for treatment 4 were the least variable of any treatment. Also, the *a* and *b* values for this treatment were found to be among the highest under each condition studied. These readings are probably due, in part, to the higher fat content in the lean of animals on treatment 4.

Shown in table 2 is the average amount of total pigment and myoglobin determined for each treatment. The total pigment values after storage are lowest for treatments 4 and 6. Only treatments 2 and 4, however, are significantly different from each other at the 0.05 level (L.S.D.=0.75) before storage. After storage, no significant differences were found between treatments. Moisture losses during storage apparently varied inversely with the fat content; therefore, the proportion of total pigment present

TABLE 2. CONCENTRATION IN MILLIGRAMS PER GRAM OF TOTAL PIGMENT AND MYOGLOBIN IN BEEF RIBEYE MUSCLE

Treatment[a]	Total pigment		Myoglobin
	Before storage	After storage	
1	5.9	8.0	3.89
2	6.6	7.7	4.05
3	6.0	7.6	4.04
4	5.7	7.4	3.20
5	6.0	7.5	3.34
6	5.3	6.8	3.34

[a] Mean of 10 animals.

after storage tended to become more nearly equal for all treatments. Further evidence for this statement was shown when pigment concentrations calculated on a moisture-free, fat-free basis were found not to be significantly different between treatments. Myoglobin values show basically the same trends as those already mentioned above. It would thus appear that the pasture *per se* did not appreciably effect the pigment concentration in the meat, although one group of workers has reported that the increased amount of exercise involved in grazing resulted in higher pigment concentration than when animals were fed in dry lot (Shenk *et al.*, 1934). Other workers, Mitchell and Hamilton (1933) and Bull *et al.* (1942), stated that they obtained no differences between exercised and unexercised beef steers. The work of the latter two groups of workers more nearly supports the results of this study.

It is interesting to note that if the mean values listed in table 2 for myoglobin are subtracted from those of like treatments listed for total pigment before storage, an appreciable fraction of the total remains.

A portion of this remainder is hemoglobin and possibly a small quantity of other pigments. A comparison of the range of myoglobin values obtained here in milligrams per gram of fresh tissue (1.62 to 6.30) compares closely with those reported by Ginger *et al.* (1954), 2.36 to 5.41, and Shenk *et al.* (1934), 3.00 to 5.00. The values reported by Watson (1935) are higher, 6.3 to 6.7, while the average value reported by Drabkin (1950) is lower, 1.69. It should be mentioned, however, that this last value represents the analyses of only one animal. Similarly, the optical density range of 0.419 to 0.948 for total pigments reported by Husaini *et al.* (1950) compares closely with the range of 0.475 to 0.893 found in this study at the same dilution. Three methods were compared for use in determining total pigment and the values obtained were all found to be in close agreement with the values used here.

TABLE 3. GRADE AND COMPOSITION OF CATTLE CARCASSES FED VARYING AMOUNTS OF GRAIN AND GRASS

Treatment no.[a]	Grade[b]	Moisture	Fat
		%	%
1	16.3	74.1	2.5
2	16.8	73.6	2.7
3	17.9	73.8	2.3
4	18.1	73.3	3.2
5	17.6	73.3	3.0
6	18.4	73.6	3.3

[a] Mean of 10 animals.
[b] The highest numbers designate the highest grade. The coding system used is the same as proposed in the Reciprocal Meat Conference report, 1957.

As a result of the discrepancy between total pigment values and myoglobin, it was concluded that either more hemoglobin was present than the 5 to 10% reported (Husaini *et al.*, 1950; Shenk *et al.*, 1934), or that a large percentage of the myoglobin was lost during its extraction from the solution containing the total pigments. Data will be published elsewhere which indicate that the latter is generally the case. The method used in this study is the determination of myoglobin, therefore, is considered more adaptable for obtaining relative values than for absolute values.

For purposes of comparison, table 3 shows the average grade, moisture and fat composition for each of the treatments. In general, the cattle receiving the highest level of grain supplementation produced meat with the least moisture, the most fat and the highest grades. The grades represented within each treatment 1 through 6, respectively, were as follows: 2 G+, 3 Go, 3G— and 2 So; 1 G+, 7Go, 1 G— and 1 S+; 2 C—, 4 G+ and 3 Go; 2 C+, 2 Co, 1 C—, 1G+, 2 Go and 3 G—; 2 C—, 3 G+, 4 Go and 1 G—; 3 Co, 1 C—, 4 G+, 1 Go and 1 G— (+, o and — represent the high, average and low one-third of each grade, respectively.

The letters C, G and S represent in order the choice, good and standard grades).

One steer was removed from treatment 3 and placed on treatment 4 because he would not eat the fat in the former ration. Except for the added fat these two treatments were the same. The fat in the ration apparently limited the total feed intake and because of this the carcass quality for treatment 3 was below that of treatment 4. The lower quality carcasses produced on treatment 5 were probably a result of withholding the 1.5 lb. of grain per cwt. until 3 months after the experiment was started. The proportionately larger amount of grain fed thereafter, was not of sufficient advantage for these cattle to overtake those fed on treatments 4 and 6. With the exceptions of the factors cited above, all treatments produced quality of meat consistent with the amount of grain consumed. However, if the same amount of fat had been produced in the meat by all treatments, it is unlikely that other quality factors would show a difference.

Moisture was not observed to accumulate in blisters on the ribeyes and at no time was there an accumulation of moisture on any portion of the meat surface. The top one-half inch thick cross-section containing the exposed surface of the lean was found to contain a low moisture content for representative samples of all treatments. The two adjacent sections contained more moisture, but were nearly equal in amounts. Since no unusual moisture pattern was detected, the moisture content of the remaining stored samples was not determined. Thus, it was concluded that the process of moisture migration and loss in the meat was not appreciably different between the treatments of this study.

The amount of yellow color in the fat was expressed on an optical density per gram basis. For treatments 1 through 6, respectively, the values were as follows: 0.0136, 0.0124, 0.0128, 0.0125, 0.0117 and 0.0045. Analysis of variance conducted on all values indicated a significant difference at the 0.01 level between treatment 6 and each of the other treatments. Although there was no statistical difference between any of the other treatments, it may be noted from the values listed above that less of the yellow pigment was found when more grain was fed in the ration. No difference in the storage qualities of the fat by treatments could be detected.

Summary

Sixty steers were fed six different rations composed of varying amounts of grain and grass. A sample from each carcass was analyzed for moisture, fat, myoglobin, total pigment, color reflectance of lean and yellow pigment in the fat. Significant differences were found between certain treatments for all measurements involving color of lean and fat. The differences in color of lean were due to varying amounts of fat and moisture rather than to a difference in the quantity of pigments present. With respect to color all

samples aged equally well during a 7-day storage period at 35° F. The brightest colored samples prior to storage, however, were also the brightest after storage.

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COMPARATIVE PROCEDURES FOR MEASURING THE PHOSPHORUS REQUIREMENT OF CATTLE

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MANY workers (Henderson and Weakley, 1930; Van Landingham *et al.*, 1935; Huffman *et al.*, 1933; Kleiber *et al.*, 1936; Beeson *et al.*, 1941; Long, *et al.*, 1957) have investigated the phosphorus requirement of cattle. These workers determined the effect of graded levels of dietary phosphorus upon one or more of the following measurements: Weight gain, plasma inorganic phosphorus level, efficiency of feed conversion, percentages of ash, calcium and phosphorus in bone, and phosphorus balance. Recent advances in radioisotope procedures offer supplementary criteria for measuring the phosphorus requirement of farm animals (Kleiber *et al.*, 1951; Hansard, 1952; Lotz *et al.*, 1952; Comar *et al.*, 1953). Thus, it seemed desirable to investigate the phosphorus requirement of young cattle by conventional and radioisotope procedures for measuring animal response to graded levels of dietary phosphorus.

Experimental Procedures

Eighteen grade Hereford steers weighing 320 to 400 lb. and about 10 months of age were individually fed a basal ration (0.12% phosphorus) for 47 days. They were then divided on the basis of weight and age into three groups each of six animals and individually fed their respective rations for a 70-day experimental period. Group 1 received the basal ration plus enough supplemental phosphorus to supply 1.5 gm. of phosphorus per 100 lb. body weight daily, while those in groups 2 and 3 received the basal ration plus 2.0 and 2.5 gm. of phosphorus per 100 lb. of body weight daily, respectively. The supplemental phosphorus was provided by a mixture of reagent grades dibasic calcium phosphate and calcium carbonate. These supplied 3.75 parts of calcium to one part of phosphorus, the ratio of these elements in the basal ration. The percentage composition of the basal ration was: Cottonseed hulls, 36.0; dried beet pulp, 27.0; dehydrated

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alfalfa meal, 9.0; dextrose, 22.7; feed grade urea, 1.0; sodium chloride, 0.5; corn gluten meal, 3.7; and vitamin A and D supplement, 0.1. Each animal also received 5 mg. of diethylstilbestrol in his feed daily.

The cattle were stanchioned inside a barn and individually fed twice daily all the feed they would consume within one and one-half hours. Daily feed consumption records were kept on all animals. The amount of supplemental inorganic phosphorus added daily to the ration of each steer varied inversely with the amount of feed consumed during the previous day. The procedure made it possible, with a lag of one day, to supply the designated level of phosphorus to all animals regardless of feed intake. The animals were weighed initially and at 7-day intervals during the experimental period. Each weighing was preceded by a 12-hour "shrink" period during which time food and water were not available to the animals. Adjustments in phosphorus allowance for each animal on the basis of body weight were made weekly and were based upon the most recent weight. Blood samples were taken by jugular puncture when the animals were weighed.

Twenty-eight days after the animals were first given the experimental rations, four animals from each group were transferred to metabolism units for balance studies. Each animal was fed 3629 gm. of feed daily during respective 14-day preliminary and 7-day collection periods. At the beginning of the collection period, two of the animals receiving each ration were given a single dose of one millicurie of phosphorus-32 orally while the other two received the same dosage of phosphorus-32 intravenously. The comparative balance and isotope dilution procedures (Hansard, 1956) were used to calculate the fecal endogenous phosphorus. Six other animals, two in each treatment group, were given intraperitoneally one millicurie of calcium-45 when the animals were first put on the experimental rations. These animals were slaughtered at the end of the experiment and the left femur and right metatarsus removed for bone growth measurements from autoradiographs (Lotz *et al.*, 1952). In addition, the right femur, left metatarsus, right metacarpus and 7th rib were removed from these animals and samples taken from the middle section of each for determination of percentage ash, calcium and phosphorus. Aliquots of liver, kidney, heart and biceps femoris muscle were taken for measuring phosphorus content. Analysis of feed, excreta, plasma and various tissues for calcium and inorganic phosphorus were made by modification of methods of Clark and Collip (1925) and Fiske and SubbaRow (1925), respectively. The statistical analyses of the data were conducted by the analysis of variance method (Snedecor, 1956) and the multiple range test (Duncan, 1955).

Results and Discussion

When response criteria were weight gain, feed consumption or feed efficiency (table 1) responses to graded levels of phosphorus did not differ

significantly from linearity ($P<.05$), and in each case linearity accounted for more than 90% of the treatment variation, suggesting that 2.0 gm. of phosphorus per 100 lb. body weight did not meet the phosphorus requirement for these functions. If bone growth and plasma inorganic phosphorus data were the response criteria, however, it was found that differences between animals in groups 2 and 3 were small and the responses to phosphorus levels differed significantly from linearity ($P<.05$), indicating that 2.0 gm. of phosphorus per 100 lb. body weight met the phosphorus requirement for these functions. These results are in agreement with those of Burroughs *et al.* (1956) and suggest that phosphorus requirement for weight gain and feed response is greater than for bone growth or maintenance of plasma inorganic phosphorus level. Variations in the day-by-day feed consumption of these individually fed animals bear on this point. Such variations were observed to be cyclic in nature; animals in group 1 completing a cycle in about 10 to 12 days while those in groups 2 and 3 were completing cycles in about 8 to 9, and 6 to 7 days, respectively. Calculations of percentage ration phosphorus revealed that groups 1, 2 and 3 consumed rations averaging 0.14, 0.17 and 0.20% phosphorus, respectively, during the entire trial. Calculations of percentage ration phosphorus were also made from feed records of animals in group 3 when feed intakes were at the lowest and highest points within the feed consumption cycle, and it was found that the cattle were receiving at these two points 0.27–0.31% and 0.16–0.18% ration phosphorus, respectively. These animals responded rapidly to the high level of ration phosphorus and within several days had again reached the peak of the cycle. The build-up in percentage ration phosphorus was slower in groups 1 and 2 than in group 3 and may have accounted for the longer time required for these animals to complete the cycle.

TABLE 1. EFFECT OF DIETARY PHOSPHORUS LEVEL UPON WEIGHT GAIN, FEED CONSUMPTION, FEED EFFICIENCY, BONE GROWTH AND PLASMA INORGANIC PHOSPHORUS LEVEL OF CATTLE

Item	Group 1	Group 2	Group 3
Phosphorus per 100 lb. body weight daily, gm.	1.5	2.0	2.5
Daily gains, lb.	1.2	1.5*	1.7**
Daily feed consumption, lb.	9.4	10.3*	11.0**
Feed efficiency, lb.	7.9	6.9*	6.5**
Bone growth			
Metatarsus (proximal end), mm.	5.4	6.5*	6.6*
Femur (proximal end), mm.	5.4	6.7*	6.7*
Femur (distal end), mm.	6.3	8.4*	8.6*
Change in plasma inorg. phosphorus, mg. %	−1.0	0.0*	+0.2*

* Different from Group 1 ($P<.05$).
** Different from Group 1 ($P<.01$).

Phosphorus is so largely concerned in the utilization of the main organic nutrients (Mitchell, 1947) and in the microbial digestion of cellulose (Anderson *et al.*, 1956) that it is logical to assume that the requirements for this element by cattle should parallel the amounts of the organic nutrients consumed. As the feed consumption curves of all animals were cyclic, the results of this trial lend support to this idea and suggest that the phosphorus requirements of growing cattle can be best expressed as a percentage of dry feed consumed. The results of Burroughs *et al.* (1956) also bear out this point.

TABLE 2. EFFECT OF DIETARY PHOSPHORUS LEVEL UPON THE DIGESTIBILITY AND RETENTION OF CALCIUM AND PHOSPHORUS BY CATTLE

Item	Group 1	Group 2	Group 3
Phosphorus per 100 lb. body weight daily, gm.	1.5	2.0	2.5
Calcium			
Intake, gm.	22.4	30.6	38.3
Excretion			
Feces, gm.	20.1	26.5*	30.7**
Urine, gm.	0.8	0.3*	0.1**
Apparent digestibility, %	11.1	13.4	19.8*
Retention, %	7.5	12.4	19.8*
Phosphorus			
Intake, gm.	6.0	8.1	10.1
Excretion			
Feces, gm.	4.2	4.7*	5.0**
Urine, gm.	0.2	0.2	0.3
Apparent digestibility, %	30.0	42.0*	50.5**
Retention, %	26.7	39.5*	47.1**
Endogenous, gm.	2.3	2.6	2.8
True digestibility, %	68.4	73.5	78.0

* Different from Group 1 ($P<.05$).
** Different from Group 1 ($P<.01$).

When response criteria were apparent digestibility or net retention of dietary phosphorus (table 2), responses to graded levels of phosphorus did not differ significantly from linearity ($P<.05$), the linearity accounting for 80% of the treatment variation in each case. These results are in agreement with those obtained when response criteria were daily gains, daily feed consumption or efficiency of feed utilization. As feed intake in all animals was restricted to 8 lb. daily during the metabolism phase, it is possible that the maximum expression of the value of added phosphorus to animals in group 3 was not obtained. It should be noted that average daily feed intakes for the entire trial were 9.4, 10.3 and 11.0 lb. for groups 1, 2 and 3, respectively.

Except for increased urinary excretion of calcium by animals in groups 1 and 2, the calcium metabolism data appear to parallel the percentage

digestibility and net retention of phosphorus. Hansard and Plumlee (1954) observed increased urinary phosphorus excretion when the intake of calcium was low and suggested that when there is insufficient calcium present in the blood for bone calcification to proceed a part of the excess phosphorus is excreted via the urine. From the results of the present experiment, it would also appear that a part of the excess calcium is excreted via the urine if an insufficient amount of phosphorus is present. Phosphorus levels did not significantly affect fecal endogenous or true digestibility of phosphorus. Visek *et al.* (1953) also noted that in short time experiments with cattle, wide variations in calcium intake had very little effect upon fecal endogenous calcium excretion. Analyses of dry fat-free bones for ash, calcium and phosphorus revealed that treatment differences were insignificant. This was also true when the soft tissues were considered; however, it is of interest to note that without exception tissues from animals in groups 2 or 3 contained slightly more phosphorus than those from animals in group 1.

Even though the primary purpose of the present experiment was to compare responses to graded levels of phosphorus when various response criteria were employed, it is of interest to compare the phosphorus requirement of growing cattle found here with that found by other workers. When the common response criteria were feed consumption, gains or efficiency of feed utilization, the results of the present experiment indicate a phosphorus requirement higher than that reported by Beeson *et al.* (1941). Certain differences in experimental animals bear on this point. Their cattle were heavier (averaging 450 vs. 350 pound initially) and were fed for a longer period of time (120 vs. 70 days). In addition, the cattle in the present trial were given 5 mg. diethylstilbestrol daily and were individually fed, whereas their cattle received no diethylstilbestrol and were group-fed. Also, in the experiment of Beeson *et al.* (1941) phosphorus was fed as a percentage of the dry weight of feed rather than on the basis of body weight. As pointed out previously, feed consumption curves in the present trial were cyclic in nature. If as suggested by Beeson *et al.* (1941) the phosphorus requirement of the cattle was 0.18% ration phosphorus, the animals in group 3 when at the peak of the feed consumption cycle were receiving less phosphorus than needed. Conversely, when feed consumption dropped, the percentage of ration phosphorus increased and during this phase of the cycle the requirement of the animal was exceeded. As excess phosphorus performs no useful function, it is apparent that the method of feeding used in the present trial could identify a phosphorus requirement above that needed for efficient utilization of the organic nutrients and for other functions. These results indicate that in phosphorus requirement experiments growth and feed response may be used as criteria indicative of the over-all functions of this element and that the phosphorus requirement of cattle can be expressed best as a percentage of dry feed consumed.

Summary

Eighteen grade Hereford steers averaging 350 lb. initially were used to compare results using various response criteria when three levels of phosphorus were fed. Six animals in each group were fed a low-phosphorus basal diet (0.12%) plus enough supplemental phosphorus to supply each animal with 1.5, 2.0 and 2.5 gm. of phosphorus per 100 lb. body weight, daily. These designated levels of phosphorus were maintained regardless of amount of feed consumed. When response criteria were weight gains, feed consumption, efficiency of feed utilization, percentage digestibility of phosphorus or percentage net retention of phosphorus, 2.0 gm. of phosphorus per 100 lb. body weight did not meet the phosphorus requirement of these animals. When response criteria were bone growth as measured by autoradiographs and plasma inorganic phosphorus level, 2.0 gm. of phosphorus per 100 lb. body weight met the phosphorus requirement. Analyses of selected bones and soft tissues for calcium and phosphorus content revealed no effect of levels of phosphorus.

Day-by-day feed consumption records revealed that feed consumption curves in all animals were cyclic in nature and that feed consumption was related to the phosphorus content of the ration. Implications of this observation as related to response criteria and methods of determining the phosphorus requirement of cattle are discussed.

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ORAL AND PARENTERAL ADMINISTRATION OF IRON IN THE PREVENTION AND TREATMENT OF BABY PIG ANEMIA [1, 2]

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THE effectiveness of single or infrequently repeated doses of oral iron in protecting baby pigs against anemia is open to question. Iron absorption is affected by the size of the dose (Granick, 1949; Stewart *et al.*, 1950), the state of iron repletion (McCance and Widdowson, 1937), and the presence of phosphates (Davidson *et al.*, 1933; Hegsted *et al.*, 1949) and reducing substances (Bergeim and Kirch, 1949; Moore *et al.*, 1939; Powell, 1944; Tompsett, 1940). At best, absorption efficiency is very low. For this reason, numerous experiments have been conducted in a search for forms of iron adaptable to parenteral administration (Holly, 1951; Horrigan *et al.*, 1950; Sinclair and Duthie, 1950; Scott and Govan, 1951). The ideal compound would be non-toxic and capable of releasing iron to the hematopoietic system at a rate commensurate with normal hemoglobin formation.

In 1952, London and Twigg at the Benger Laboratories, Ltd. in Holmes Chapel, Cheshire, England, successfully produced an iron-dextran complex for intramuscular treatment of hypochromic anemia in man. Dextran is a bacterial polysaccharide composed of D-glucopyranose units linked primarily in the 1–6 position but with some 1–4 bridges. The dextran employed in this complex was said to be of relatively low molecular weight although the exact composition is not yet known (1/6/58).

Subsequent publications (Barber *et al.*, 1955; Brownlie, 1955; Kernkamp, 1957; Martin *et al.*, 1955; McDonald *et al.*, 1955) indicated that this complex would be very useful in preventing and treating baby pig anemia.

It was our objective to study the effect of various treatments, including parenteral iron-dextran administration, upon hematology and weight gains of pigs.

Experimental Procedure

One hundred seventy-five Duroc and cross-bred pigs were used. They were farrowed between March 15 and 28 of 1957 and were housed on

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concrete during the course of the experiment. The pigs were allotted to treatment at random from litter outcome groups. At approximately 3 weeks of age they were given access to creep feed.

Treatments consisted of: (1) No iron supplementation; (2) an iron-copper-cobalt tablet administered orally, supplying 292 mg. of reduced iron, 21.6 mg. of copper sulfate and 2.8 mg. of cobaltous sulfate; (3) two ml. of an iron-dextran solution administered intramuscularly in the semitendinosus, semimembranosus, or biceps femoris, supplying 100 mg. of ferrous iron; (4) two ml. of the iron-dextran solution intramuscularly plus 5 ml. of a hematopoietic vitamin solution intraperitoneally. This supplied 1 g. of nicotinamide, 100 mg. of pyridoxine, 10 mg. of folic acid and 0.1 mg. of vitamin B_{12}.

The oral tablet was administered 3 or 4 days after birth and again at 3 weeks. The iron-dextran and vitamins were administered only once at 3 or 4 days of age.

Complete hematology was performed weekly on 40 of the animals until 5 weeks of age. Blood was obtained from the anterior vena cava. The hematological study included enumeration of erythrocytes, reticulocytes, and leucocytes, differential leucocyte counts, hemoglobin and hematocrit determination, total serum protein determination and serum protein electrophoresis. The remaining pigs until 5 weeks old were bled biweekly from an ear vein for the determination of hemoglobin and hematocrit.

All pigs were weighed weekly and death losses were noted. Erythrocytes and leucocytes were counted in duplicate from a single filling of a "zero error" Hellige pipette. The diluting fluids were 0.85% (W/V) NaCl for erythrocytes and 3% (V/V) glacial acetic acid for leucocytes. Cells were counted in a hemocytometer with Neubauer ruling and a National Bureau of Standards certified cover glass. Acceptable duplicate counts differed no more than 8% of the lower count.

Blood smears for differential counts were prepared with Wright's stain buffered at pH 6.8. Two hundred cells were counted. Reticulocytes were enumerated per 1000 erythrocytes in blood smears vitally stained with brilliant cresyl blue and counterstained with Wright's stain.

Hemoglobin was determined by the cyanmethemoglobin method of Crosby *et al.* (1954).

Hematocrit was determined by the micro-method described by McGovern *et al.* (1955). Blood samples were centrifuged for 5 minutes at 10,000 r.p.m. in an International "Hemacrit" centrifuge. This method produced consistently lower values (Wintrobe hematocrit $-1.44\% \pm .10$) in 102 comparisons with the Wintrobe procedure in which the tubes were spun for 30 minutes at 3000 r.p.m. in the International Size 1 centrifuge.

Total serum protein was determined by the biuret procedure accepted by the American Association of Clinical Chemists (Reiner, 1953). Pooled porcine serum was standardized by macro-Kjeldahl methods.

Electrophoretic separation of serum proteins was accomplished on a Spinco Model R paper electrophoresis system (Spinco Technical Bulletin 6026A).

At 5 weeks of age, 21 pigs which had received no iron were divided among the four previously described treatments. Hemoglobin and hematocrit determinations were performed 1 week and 3 weeks after treatment.

Results and Discussion

The effect of treatment on weight gains, hemoglobin and hematocrit of all pigs is illustrated in figures 1, 2 and 3. Results of complete hematology performed on 40 pigs are presented [4] in tables 1, 2 and 3.

Body weight was significantly less ($P<.05$) at 5 weeks for pigs receiving no iron treatment.

In general, pigs receiving no supplemental iron or the oral tablet began to exhibit symptoms of anemia at 10 days of age. This was reflected primarily in reduced hemoglobin, hematocrit, and erythrocyte count. The mean corpuscular volume was similarly reduced although mean corpuscular hemoglobin concentration was not significantly affected.

Intraperitoneal injection of vitamins appeared to retard the drop in percent neutrophils which normally occurs from birth to 5 weeks. However, no group receiving a placebo intraperitoneally was studied and this effect cannot be ascribed directly to the vitamins.

Total serum protein was elevated at 10 and 17 days in pigs receiving no treatment. The percent gamma globulin was significantly greater at 31 days in pigs receiving iron-dextran plus vitamins than in those not treated.

The effectiveness of various treatments in alleviating anemia in 5-week-old pigs is illustrated in figures 4 and 5. The iron-dextran injections produced a faster and more rapid response than did the oral tablet. Improvement was also noted in the untreated pigs and this was thought to be associated with increased consumption of creep feed.

Twenty-seven baby pigs raised concurrently from 4 days of age on a synthetic milk had considerably higher hemoglobin (figure 6) and hematocrit values throughout the 5-week period than any of the treatment groups raised on the sow. These pigs had received iron-dextran intramuscularly or iron-dextran plus intraperitoneal vitamins at 4 days and their diet contained 228 ppm of iron as $FeSO_4 \cdot 7H_2O$. It appeared that one injection, providing 100 mg. of iron, did not entirely match the capacity of the pig's hematopoietic system for hemoglobin synthesis.

As a practical means of preventing and treating baby pig anemia, intramuscular iron-dextran was very efficacious, producing a peak of hematological values approximately 2 weeks after injection.

[4] Standard error of the means in these three tables are on file in the Department of Animal Husbandry.

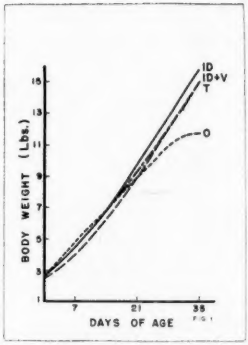

Figure 1. Effect of treatment at 3 to 4 days of age on body weight gains (175 pigs). No supplementation=O; Fe-Cu-Co oral tablet=T; iron-dextran intramuscularly =ID; iron-dextran plus intraperitoneal-vitamins=ID+V.

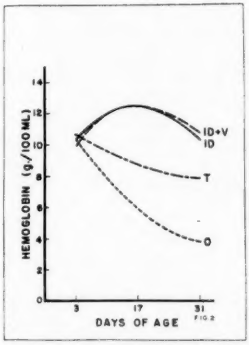

Figure 2. Effect of treatment at 3 to 4 days of age on hemoglobin (175 pigs).

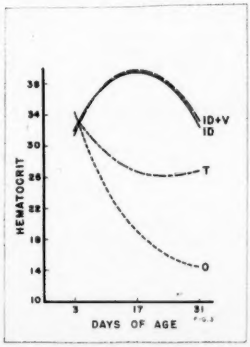

Figure 3. Effect of treatment at 3 to 4 days of age on hematocrit (175 pigs).

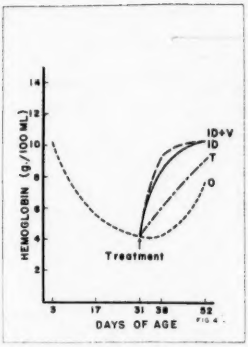

Figure 4. Effect of treatment at 31 days of age on hemoglobin (21 pigs).

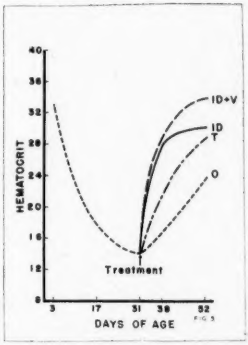

Figure 5. Effect of treatment at 31 days of age on hematocrit (21 pigs).

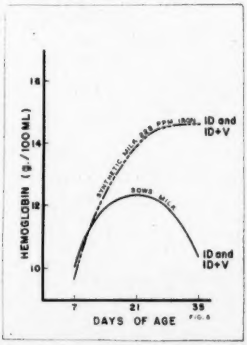

Figure 6. Comparison of hemoglobin response between pigs receiving either a synthetic milk (27) or nursing the sow (175).

TABLE 1. EFFECT OF TREATMENT ON ERYTHROCYTES

Treatment and No. of pigs	No supplemental iron 7	Fe-Cu-CO tablets 16	Iron-dextran 10	Iron-dextran + B-vit. 7
Days of age		Hemoglobin, gm./100 ml. blood		
3	10.16	10.83	9.74	9.63
10	6.40	9.80[a]	11.12[a]	10.47[a]
17	5.17	7.92	12.41[b]	11.47[b]
24	4.05	8.04[a]	10.69[a]	11.41[a, d]
31	5.23	6.65	9.81[b]	9.55[b]
		Hematocrit, %		
3	29.1	32.7	30.0	30.0
10	20.6	31.4[a]	36.9[a, d]	33.8[a]
17	16.1	23.6[a]	34.9[a, b]	36.9[a, b]
24	12.2	24.6[a]	31.9[a, b]	34.0[a, b]
31	13.2	21.3[a]	29.0[a, b]	28.9[a, b]
		Erythrocytes, millions/cubic mm.		
3	4.35	4.60	4.80	4.78
10	3.59	4.73[a]	4.71[a]	5.09[a]
17	3.26	4.17[c]	5.08[a, d]	4.86[a]
24	2.29	4.36[a]	5.00[a]	4.89[a]
31	3.04	3.98[c]	5.39[b]	5.12[b]
		Reticulocytes, % of erythrocytes		
3	6.7	5.3	4.6	5.5
10	6.2	6.1	6.4	7.3
17	8.3	7.7	8.8	11.1[c]
24	5.5	5.5	3.6	6.4
31	7.1	6.1	5.0	3.4
		Mean corpuscular volume, cubic microns		
3	70.8	72.8	63.6	67.0
10	57.4	67.9[c]	77.9[a]	67.9[c]
17	48.5	56.9[c]	65.7[a, d]	77.1[b]
24	49.4	50.1	65.0[b]	71.0[b]
31	44.2	54.6[c]	56.6[c]	57.9[c]
		Mean corpuscular hemoglobin concentration, %		
3	33.5	32.4	32.6	32.2
10	31.6	31.3	30.2	31.1
17	29.6	31.9	35.8[c]	30.9
24	31.4	32.3	33.5	33.5
31	30.7	31.2	33.9	32.9

[a] Significantly greater than least value ($P<.01$).
[b] Significantly greater than 2 least values ($P<.01$).
[c] Significantly greater than least value ($P<.05$).
[d] Significantly greater than 2 least values ($P<.05$).

TABLE 2. EFFECT OF TREATMENT ON LEUCOCYTES

Treatment and No. of pigs	No supplemental iron 7	Fe-Cu-CO tablets 16	Iron-dextran 10	Iron-dextran + B-vit. 7
Days of age		Leucocytes/cubic mm.		
3	6714	7196	6529	6452
10	7534	10485	9632	11253
17	6687	6613	6694	8002
24	6921	7328	9362	11685
31	10966	9771	10825	12136
		Lymphocytes, %		
3	51	48	52	50
10	68[a, d]	58	62	55
17	67[c]	72[a]	72[a]	55
24	81	78	74	72
31	84[c]	72	73	67
		Monocytes, %		
3	2	3	3	3
10	2	3	2	2
17	2	2	3	3
24	3	3	5[d]	5[d]
31	2	4	7[a, d]	5
		Eosinophils, %		
3	0.9	0.8	0.8	0.8
10	.1	1.4[c]	.4	.5
17	.2	1.9[a]	1.0	1.3
24	.6	.9	.9	.7
31	.2	.7	.2	.8
		Basophils, %		
3	0.2	0.2	0.4	0.3
10	.2	.2	0.0	.2
17	0.0	0.0	.1	0.0
24	.2	0.0	0.0	.1
31	0.0	.1	.2	0.0
		Total neutrophils, %		
3	51	48	44	45
10	30	38	35	42[c]
17	31	25	24	40[d]
24	15	19	20	22
31	13	24	19	27[c]
		Non-segmented neutrophils, %		
3	0.1	0.1	0	0
10	0.0	0.0	0	0
17	0.0	0.0	0	0
24	0.0	0.0	0	0
31	0.0	0.0	0	0
		Metamyelocytes, %		
3				
10				
17		None observed		
24				
31				
		Myelocytes, %		
3				
10				
17		None observed		
24				
31				

[a] Significantly greater than least value ($P<.01$).
[b] Significantly greater than 2 least values ($P<.01$).
[c] Significantly greater than least value ($P<.05$).
[d] Significantly greater than 2 least values ($P<.05$).

TABLE 3. EFFECT OF TREATMENT ON SERUM

Treatment and No. of pigs	No supplemental iron 7	Fe-Cu-CO tablets 16	Iron-dextran 10	Iron-dextran + B-vit. 7
Days of age	Total serum protein, gm./100 ml. serum			
3	4.79	4.20	4.22	4.30
10	5.28[d]	4.51	4.51	5.07
17	5.04[c]	4.51	4.27	4.79
24	5.15	4.85	4.66	4.69
31	5.08	4.99	4.63	4.68
	Serum albumin, % of total serum protein			
3	13.9	13.7	14.0	14.9
10	35.4	36.1	38.9	33.9
17	41.2	44.2	44.0	45.8
24	45.7	43.6	43.4	46.1
31	43.5	45.6	44.6	43.9
	Serum alpha globulin, % of total serum protein			
3	32.6	37.8	36.4	33.8
10	30.1	29.2	27.2	30.1
17	27.0	26.8	25.3	25.2
24	25.4	26.1	27.7	26.0
31	29.1	27.4	27.8	26.0
	Serum beta globulin, % of total serum protein			
3	19.8	18.3	18.5	17.7
10	21.0	20.2	18.8	21.7
17	21.0	20.0	21.6	19.7
24	21.0	21.0	19.1	19.5
31	21.7	21.0	19.5	18.5
	Serum gamma globulin, % of total serum protein			
3	33.8	30.2	31.0	33.6
10	13.5	15.1	15.2	15.4
17	10.7	9.0	9.1	9.2
24	7.9	9.3	9.8	8.4
31	5.6	6.1	8.0	11.7[b]

[a] Significantly greater than least value ($P<.01$).
[b] Significantly greater than 2 least values ($P<.01$).
[c] Significantly greater than least value ($P<.05$).
[d] Significantly greater than 2 least values ($P<.05$).

Summary

One hundred seventy-five baby pigs were given either no iron supplementation, oral iron tablets, or an intramuscular injection of iron-dextran with or without an intraperitoneal injection of vitamins. Hematological evidence established the superiority of intramuscular iron-dextran. No improvement was achieved by adding an intraperitoneal injection of certain vitamins.

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MAGNESIUM REQUIREMENT OF THE PIG

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MAGNESIUM has been shown to be essential in the diets of young rats (McCollum and Oren, 1931; Kruse *et al.*, 1933; Tufts and Greenburg, 1938), mature rats (Barron *et al.*, 1948), guinea pigs (Roine *et al.*, 1949), dogs (Orent *et al.*, 1932), chicks (Almquist, 1942; Bird, 1949), ducks (Van Reen and Pearson, 1953), rabbits (Kundel and Pearson, 1948) and calves (Duncan *et al.*, 1935). Magnesium has been assumed to be essential in the ration of swine and is routinely added to semipurified rations for this species but is not usually added to practical rations. Studies have not been reported on either the deficiency symptoms or the quantitative requirement of magnesium for swine.

The objectives of these three experiments were (1) To determine if magnesium is required in the ration of swine weaned at either three or nine weeks of age; (2) to characterize the deficiency symptoms; and (3) to determine the quantitative magnesium requirement of pigs weaned at either 3 or 9 weeks of age (Mayo *et al.*, 1957).

Experiment I

Experimental

During February of 1957, 24 Duroc barrows and gilts 3 weeks of age and averaging 10.5 lb. in weight were allotted to four treatments according to sex and liveweight. Table 1 shows the composition of the two semipurified basal rations used in this experiment. One basal contained blood fibrin and the other contained Drackett C-1 assay protein as the source of protein; the two rations contained 35 and 70 p.p.m. of elemental magnesium, respectively. The four treatments were: Blood fibrin basal (lot 1), blood fibrin basal plus 343 p.p.m. of magnesium (lot 2), Drackett basal (lot 3) and Drackett basal plus 343 p.p.m. of magnesium (lot 4). Thus the total magnesium in the ration of lots 1, 2, 3 and 4 was 35, 378, 70 and 413 p.p.m., respectively. U.S.P. grade magnesium carbonate $(MgCO_3)_4Mg(OH)\cdot 5H_2O$ containing approximately 26% magnesium was the source of added magnesium. The experiment consisted of three phases, depletion, repletion and a second depletion. At the termination of the first depletion period on the 21st day, the six surviving pigs in lots 1 and 3 (3 in each lot) were placed on magnesium supplemented rations fed lots 2 and 4, respectively. After 14 days on the

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 1262. Purdue University Agricultural Experiment Station.

repletion rations, the six pigs in lots 1 and 3 were switched back to the original treatments of beef blood fibrin and Drackett protein basal rations, respectively. This second depletion period was for 28 days. All six pigs of each treatment were group fed for the first two weeks; thereafter, males and females were maintained separately, three per pen. The pigs were

TABLE 1. PERCENT COMPOSITION OF THE SEMIPURIFIED BASAL RATIONS

Ingredient	Experiment			
	I	I	II[a]	III
Isolated soybean protein[b]	——	25.00	26.00	20.00
Beef blood fibrin	25.00	——	——	——
Corn sugar (cerelose)[c]	30.94	30.94	30.44	33.44
Corn starch[c]	30.73	30.73	30.23	33.23
Corn oil[c]	5.00	5.00	5.00	5.00
Celluflour	3.00	3.00	3.00	3.00
DL-methionine	0.25	0.25	0.25	0.25
Liver extract	0.50	0.50	——	——
Liver meal	——	——	0.50	0.50
$CaCO_3$	0.50	0.50	0.50	0.50
$CaHPO_4.2H_2O$	2.00	2.00	2.00	2.00
KH_2PO_4	0.50	0.50	0.50	0.50
Iodized salt	0.50	0.50	0.50	0.50
Trace mineral mixture[d]	0.14	0.14	0.14	0.14
Vitamin-antibiotic mixture[e, f]	0.44	0.44	0.44	0.44
Total	100.00	100.00	100.00	100.00

[a] Liver extract was fed for the first 14 days.
[b] Drackett C-1 Assay Protein.
[c] Corn Products Refining Company, Argo, Illinois.
[d] Trace minerals added in grams per 100 pounds of ration: $FeSO_4 \cdot 7H_2O$, 49.0; $CuSO_4$, 1.28; ZnO, 2.825; $MnSO_4 \cdot H_2O$, 5.125; and CaF_2, 1.0.
[e] Vitamins added in milligrams per pound of ration: Thiamine HCl, 3.0; riboflavin, 4.0; niacin, 15.0; Ca pantothenate, 10.0; pyridoxine, HCl, 1.50; choline chloride, 600.0; para amino benzoic acid, 50.0; biotin, 0.10; inositol, 50.0; folic acid, 2.0; ascorbic acid, 50.0; mixed tocopherols, 15.0; 2 methyl-1,4 napthoquinone, 1.0; vitamin B_{12}, 20.0 micrograms per pound; vitamin A palmitate, 2000 I.U. of vitamin A per pound; vitamin D_3, 400 I.U. per pound; corn starch as carrier, 1032 mg. per pound.
[f] Chlortetracycline HCl, 25 mg. per pound of total feed.

housed in a heated barn and confined to concrete pens 4′ x 8′ bedded with corn cobs. All drinking water was treated with a zeolite softener which lowered the magnesium content of the water from 355 to 1.6 p.p.m. Pigs were weighed individually every 7 days and weekly feed consumption was recorded for each group of pigs. Blood serum magnesium was determined each weigh day by the colorimetric method of Heagy (1948). The magnesium content of each basal ration was determined by the colorimetric method described by Sandell (1950). A modification of Heagy's method was used to determine the magnesium content of the drinking water (aliquots of the drinking water were substituted for the magnesium solution

used in preparing the standards). Experimental data were analyzed by the analysis of variance as outlined by Snedecor (1956).

Results

Table 2 summarizes the growth, feed and serum magnesium data for each of the three phases. Half of the pigs which were fed the basal rations,

TABLE 2. GROWTH, FEED CONSUMPTION, FEED CONVERSION AND SERUM MAGNESIUM DATA FOR EXPERIMENT I

Item	Treatment [a]			
	Fibrin basal	Fibrin + Mg	Drackett basal	Drackett + Mg
	1st Depletion—21 days [c]			
Dietary Mg, p.p.m.	35	378	70	413
Av. daily gain, lb.	0.26	0.47	0.19	0.40
Av. daily feed, lb.	0.61	0.87	0.71	1.07
Feed/lb. gain, lb.	2.34	1.85	3.79	2.67
Serum Mg, final, mg., % [b]	0.37	1.18	0.56	1.22
	Repletion—14 days			
Dietary Mg, p.p.m.	378	378	413	413
Daily gain, lb.	0.93	1.05	0.90	0.99
Daily feed, lb.	1.80	2.00	1.87	2.29
Feed/lb. gain, lb.	1.94	1.90	2.07	2.30
Serum Mg, final, mg., %	2.01	2.26	1.66	1.76
	2nd Depletion—28 days			
Dietary Mg, p.p.m.	35	378	70	413
Daily gain, lb.	1.21	1.48	1.00	1.53
Daily feed, lb.	3.11	3.46	2.47	3.40
Feed/lb. gain, lb.	2.58	2.35	2.47	2.23
Serum Mg, final, mg., %	1.32	2.32	1.06	2.01

	Least significant differences		
	First depletion	Repletion	Second depletion
Daily gain	5%=0.11 1%=0.16	5%=0.22 1%=0.31	5%=0.32 1%=0.45
Serum Mg (mg. %)	5%=0.64 1%=0.90		5%=0.20 1%=0.27

[a] Each treatment consisted of six three week old pigs weighing 10.4 lb. initially.
[b] Average initial serum magnesium was 3.89 mg. percent.
[c] Three pigs in each basal lot died during the first depletion period.

lots 1 and 3, died during the first depletion period. Average daily gains of the surviving pigs were 0.26, 0.47, 0.19 and 0.40 lb. daily for the 35, 378, 70 and 413 p.p.m. magnesium treatments, respectively. The corresponding final serum magnesium values were 0.37, 1.18, 0.56 and 1.22 milligrams percent. These values for both growth rate and serum magnesium were significantly lower ($P<0.01$) for the 35 and 70 p.p.m. magnesium groups than for the 378 or 413 p.p.m. magnesium groups. Although the feed con-

sumption and feed efficiency data could not be reliably analyzed because of excessive mortality in lots 1 and 3, the differences due to level of dietary magnesium were obvious.

On the 17th day of the experiment deficiency symptoms appeared in lots 1 and 3. In order of appearance the following symptoms were noted: Noticeably weak front pasterns, sickled hocks, a concave bowing back of the front legs due to extreme pastern weakness, knock-knees and hocks, hyperirritability, muscular twitching, arched backs, reluctance to stand

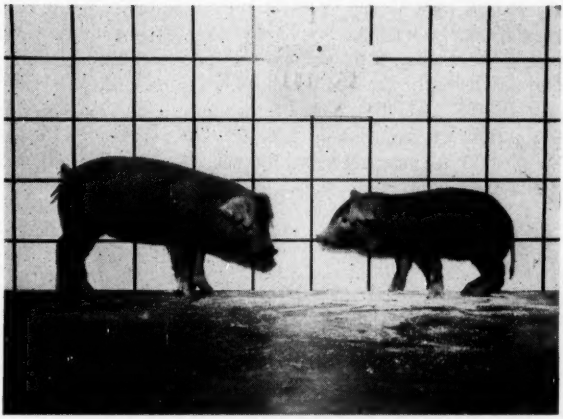

Figure 1. Magnesium deficiency in six-week old pigs. Left—fed 413 p.p.m. magnesium for 3 weeks. Right—fed 70 p.p.m. magnesium for the same period. Note extreme leg weakness of pig on the right.

and a continual shifting of the feet while standing followed by tetany and death (see Figure 1). Post mortem examinations failed to reveal any gross anatomical lesions which could be specifically associated with a magnesium deficiency in swine. Nearly all of the pigs which were autopsied had ground corn cobs in their digestive tracts indicating depraved appetite during the deficiency.

When the surviving magnesium deficient pigs of lots 1 and 3 were fed rations containing 378 and 413 p.p.m. magnesium in the repletion period, the rate of recovery was remarkable. Increased activity and feed consumption were observed within 4 hours and after 2 days most of the deficiency symptoms had disappeared. The last deficiency symptom to be corrected was the front pastern weakness, but all of the pigs in lots 1 and 3 were completely normal appearing after 4 days on the repletion rations. Daily gains and serum magnesium levels increased rapidly in lots 1 and 3 during the repletion period. The averages for this 14-day period were

similar for all four treatments, indicating a rapid return to normal when 343 p.p.m. of magnesium was added to the low magnesium rations.

In the final depletion phase, lots 1 and 3 were switched back to the low magnesium basal rations fed during the first depletion period (35 and 70 p.p.m. magnesium, respectively). After one week the serum magnesium levels of lots 1 and 3 were again significantly lower ($P<.01$) than for pigs in lots 2 and 4. The levels were 0.51, 1.60, 0.67 and 1.35 milligrams percent, respectively, for the 35, 378, 70 and 413 p.p.m. magnesium treatments. These differences were of the same magnitude at the termination of the second depletion period on the 28th day. On the 7th day of depletion deficiency symptoms appeared and became more severe until the 12th day. Thereafter, little change was noted in the deficiency symptoms and no deaths occurred. Daily gains were significantly lower ($P<.01$) for the pigs fed 35 and 70 p.p.m. of magnesium as compared to either 378 or 413 p.p.m. of magnesium. The 28-day average gains were 1.21, 1.48, 1.0 and 1.53 lb., respectively, for the 35, 378, 70 and 413 p.p.m. of magnesium treatments.

Experiment II

Experimental

Thirty-six Duroc boar pigs about 21 days old and having an initial weight of 13 lb. were first divided into three replicates on the basis of initial weight (light, medium and heavy) and subsequently into six lots on the basis of initial weight within replicates. Two pigs in the same replicate were maintained in each 4′ x 8′ indoor concrete floored pen. This method of allotting resulted in three pens per treatment and permitted replication on the basis of initial liveweight. The composition of the semi-purified basal ration differed slightly from the ones fed in Experiment I. Drackett C-1 was the source of protein and 1% more Drackett was fed (26% vs. 25%) at the expense of cerelose and corn starch; 0.5% whole liver meal was substituted for 0.5% liver extract on the 14th day of the experiment. The basal ration contained 70 p.p.m. of magnesium. The six treatments were 70 (basal), 136, 202, 268, 334 and 400 p.p.m. of magnesium fed for 42 days. U.S.P. grade magnesium carbonate again was the source of added magnesium. Instead of bedding with corn cobs, half of each pen was covered with a wooden platform. This arrangement had decided merit since no bedding was available for consumption as occurred during the first experiment. The pens were cleaned by washing the exposed back half of the pens. Management procedures were otherwise the same as those described in Experiment I.

Results

The growth, feed consumption, feed conversion, and serum magnesium data are summarized in table 3. Symptoms of magnesium deficiency were

noted in lot 1 (70 p.p.m. Mg) on the 13th day of the experiment. Serum magnesium levels on the 14th day of the feeding period were 0.71, 0.85, 1.08, 0.94, 0.99, 1.19 milligrams percent, respectively, for treatments 1 through 6. These differences were significant ($P<.01$) as shown by the F-test (L.S.D. .05=0.36; L.S.D. .01=0.49). These differences continued throughout the experiment.

TABLE 3. GROWTH, FEED AND SERUM MAGNESIUM DATA OF PIGS FED SEMIPURIFIED RATIONS CONTAINING SIX LEVELS OF MAGNESIUM IN EXPERIMENT II

Lot [a]	Mg,	Daily gain	Daily feed	Feed/lb. gain	Final serum [b] Mg	Deaths
	p.p.m.	lb.	lb.	lb.	mg. %	
1	70	0.39	0.93	2.41	Nil	5
2	136	0.56	1.52	2.70	0.62	2
3	202	0.66	1.30	1.98	0.40	1
4	268	0.67	1.48	2.21	0.49	0
5	334	0.85	1.64	1.94	0.69	1
6	400	0.87	1.74	2.01	1.02	0

Least significant differences [c]

Daily gain	5% = 0.13	Serum Mg	5% = 0.38
	1% = 0.18		1% = 0.53

[a] Each lot consisted of six three week old pigs weighing 13.3 lb. initially and fed for 42 days.
[b] Average initial serum magnesium was 2.1 mg. percent.
[c] Calculated only when F-test showed significance at less than 0.05.

Pigs fed 334 p.p.m. of magnesium or less suffered from the typical magnesium deficiency symptoms described in the first experiment. Deaths resulting from a lack of magnesium were five in lot 1, two in lot 2 and one each in lots 3 and 5. Feed consumption and conversion tended to improve with each increment of magnesium. Deficiency symptoms and deaths occurred earlier on the lower levels of magnesium. The appearance and severity of the deficiency symptoms were inversely proportional to the amount of dietary magnesium; however, the differences between 334 and 400 p.p.m. of magnesium were small. These results indicated that the magnesium requirement to prevent appearance of deficiency symptoms was greater than the requirement for maximum growth and feed conversion.

Experiment III

Experimental

Thirty-six Yorkshire barrows and gilts about 9 weeks of age and having an average weight of 45 lb. were allotted to six treatments. Allotment was made by first dividing the pigs by sex and then into three replicates

within sex on the basis of initial weight as in Experiment II. One barrow and one gilt were kept in each 4′ x 8′ indoor concrete floored pen with three pens being used per treatment. For these nine-week-old pigs the protein content of the basal ration was reduced from 20% (Experiment I) to 16% which reduced the magnesium content to 65 p.p.m. The six treatments were: 65, 153, 241, 329, 417 and 505 p.p.m. of magnesium fed for 42 days. Supplementary magnesium was supplied by U.S.P. grade magnesium carbonate. Individual weights were taken weekly and serum magnesium levels were determined bi-weekly. Wooden platforms described

TABLE 4. SUMMARY OF GROWTH, FEED CONSUMPTION, FEED CONVERSION AND SERUM MAGNESIUM DATA FOR EXPERIMENT III

Lot [a]	Mg,	Daily gain	Daily feed	Feed per lb. gain	Final serum [b] Mg
	p.p.m.	lb.	lb.	lb.	mg. %
1	65	0.99	2.70	2.73	0.75
2	153	1.38	3.55	2.57	1.08
3	241	1.71	4.40	2.57	1.65
4	329	1.72	4.05	2.35	1.72
5	417	1.75	3.98	2.28	1.92
6	505	1.74	3.99	2.30	2.06

Least significant differences [c]

Da.	5% = 0.16	Da.	5% = 0.35	Feed/lb.	5% = 0.21	Serum	5% = 0.45
Gain	1% = 0.22	Feed	1% = 0.50	Gain	1% = 0.30	Mg	1% = 0.60

[a] Each lot consisted of six nine week old pigs weighing 45 lb. initially and fed for 42 days.
[b] Average initial serum magnesium was 2.57 mg. percent.
[c] Calculated only when F-test showed significance at less than .05.

in Experiment II were used in lieu of bedding for the pens. As in the previous experiment, all drinking water was treated with a zeolite softener. At the termination of the experiment all of the pigs in lots 1 and 4 were sacrificed. One femoral condyle from each pig was ashed and analyzed for calcium, phosphorus and magnesium by A.O.A.C. methods (1955).

A summary of daily gains, feed consumption, feed conversion and serum magnesium data is shown in table 4. On the ninth day of the experiment the pigs fed the basal ration (lot 1) showed the first deficiency symptoms. Although serum magnesium determinations were made biweekly, there were no significant differences in the serum magnesium levels until the 28th day. At the termination of the experiment each increment of magnesium fed resulted in a higher serum magnesium content. These differences in serum magnesium levels were highly significant ($P<.01$). Pigs fed 417 p.p.m. of magnesium or less suffered from the deficiency symptoms. Although no deaths occurred during the experiment, the magnitude of the deficiency symptoms were inversely proportional to the amount of dietary magnesium up to 417 p.p.m. In addition to the previously observed

symptoms several pigs became "tip-toed" on the rear feet rather than sickle hocked.

Growth rate was not improved by levels of magnesium above 241 p.p.m. However, 505 p.p.m. of magnesium was required to prevent the appearance of all magnesium deficiency symptoms. Feed consumption was not governed by the level of dietary magnesium, but feed conversion and serum magnesium levels were improved with each increment of magnesium.

TABLE 5. ANALYSES OF FEMORAL CONDYLES OF SWINE FED TWO LEVELS OF MAGNESIUM IN EXPERIMENT III

Lot number	1	4
Dietary Mg (p.p.m.)	65	329
Fresh femoral condyles:		
Ash, %	21.81	23.78
Calcium, %	8.3	9.0
Phosphorus, %	3.8	4.2
Magnesium, %	0.12	0.16
Ash of femoral condyles:		
Calcium, %	38.24	36.65
Phosphorus, %	17.38	17.60
Magnesium, %	0.58	0.66
Least significant differences	5%	1%
Fresh femoral condyles:		
Percent ash	1.97	2.80
Percent calcium	0.44	0.59
Percent phosphorus	0.18	0.24
Percent magnesium	0.09	0.14

The ash, calcium and phosphorus content of the fresh femoral condyles were significantly lower ($P < .01$) in pigs fed 65 p.p.m. of magnesium than those fed 329 p.p.m. of magnesium (see table 5). Magnesium content was reduced but not significantly. However, there were no significant differences in the calcium, phosphorus or magnesium content of the bone ash between the two treatments.

Discussion

These data indicate that the element magnesium is essential in the rations of swine weaned at either 3 or 9 weeks of age. In rats (Kruse *et al.*, 1933) and dogs (Orent *et al.*, 1932) the symptom of vasodilation was characteristic of a lack of magnesium, but rabbits (Kundel and Pearson, 1948) failed to show this symptom. In these experiments vasodilation was not observed in swine. Hyperirritability of the nervous system and tetany were noted in swine suffering from a magnesium deficiency. This was

similar to deficiency symptoms reported for the rat except no running fits occurred. This finding with pigs is more in agreement with the results reported for the dog where the vasodilation, hyperexcitability and convulsions were less intense while the trophic and nutritive changes were relatively more conspicuous than in the rat. Orent *et al.* (1932) expressed the belief that these differences between the dog and rat were due to an inherently more stable nervous system of the dog. The characteristics of the basal rations tend to rule out the possibility that the leg weaknesses noted in these experiments were caused by improper calcium, phosphorus or vitamin D supplementation. The basal rations in each of the experiments contained 0.67% calcium and 0.50% phosphorus which resulted in a calcium to phosphorus ratio of 1.34. Vitamin D_3 was added to provide a total of 400 I.U. per pound of feed. Moreover, the leg weakness resulting from a lack of magnesium can be distinguished from those caused by improper calcium or phosphorus supplementation. Plumlee *et al.* (1958) reported that a deficiency of phosphorus results in crooked legs, bones so soft they could be cut with a knife and lowered femoral condyle ash. Aubel *et al.* (1941) reported that a calcium deficiency in swine resulted in failure of normal bone development and a posterior paralysis. Pigs suffering from lack of magnesium did not have crooked leg bones nor was there any evidence of "soft bones" when the deficient pigs were autopsied. No type of posterior paralysis was observed in the magnesium deficient pigs. The feeding of magnesium repletion rations to pigs suffering from a lack of magnesium resulted in an immediate recovery from leg weaknesses and a complete recovery after 4 days. Also, the rate of symptom development and recovery of pigs with magnesium supplementation were greater than has been reported in calcium, phosphorus or vitamin D deficiencies.

It is difficult to explain why the reaction of three-week-old Duroc boar pigs in Experiment II differed from that of the nine-week-old Yorkshire barrows and gilts of Experiment III. In the first two experiments with Duroc pigs weaned at 3 weeks of age, serum magnesium levels decreased at about the same time the first deficiency symptoms were noted, but in Experiment III the Yorkshire pigs weaned at 9 weeks of age exhibited deficiency symptoms on the 9th day but serum magnesium levels were not lowered until the 28th day. Some of the pigs in Experiment III were "tip-toed" on the rear feet rather than sickle hocked as in the first two experiments. Statistical analysis revealed that there was a significant difference ($P<.01$) in growth rate due to sex in Experiment III, but there was no significant sex x treatment interaction. Age, weight, magnesium stores, breeding and a combination of these with the concrete floor appear to account for these differences.

The data from all three experiments show that the amount of magnesium required to promote optimum growth was less than the amount needed to prevent the appearance of all magnesium deficiency symptoms. Obvi-

ously the age of the animals and pre-experimental treatment influenced the data relating to magnesium requirement. This is shown by the fact that the requirement for maximum growth was 334 and 241 p.p.m., respectively, for the 3- and 9-week-old pigs; and the requirement to prevent deficiency symptoms was 400 and 505 p.p.m., respectively.

Most of the commonly fed roughages and concentrates contain at least 1000 p.p.m. of magnesium on a dry matter basis, and many have three or four times this amount (Maynard and Loosli, 1956). Thus, it would seem likely that the commonly fed rations of swine can be relied upon to supply an adequate amount of magnesium without supplementation.

Summary

Three experiments were conducted with 60 Duroc pigs weaned at 3 weeks of age and 36 Yorkshire pigs weaned at 9 weeks of age to determine the quantitative requirement of magnesium for pigs. These data indicate that:

1. Magnesium is an essential nutrient in the rations of swine weaned at either 3 or 9 weeks of age.
2. A deficiency of dietary magnesium results in the following symptoms in order of appearance: Noticeably weak front pasterns, sickled hocks, a concave bowing back of the front legs due to extreme pastern weakness, knock-knees and hocks, hyperirritability, muscular twitching, arched back, reluctance to stand and a continual shifting of the feet while standing, followed by tetany and death.
3. The above symptoms were accompanied by a statistically significant reduction in growth rate, feed consumption, feed conversion and serum magnesium.
4. The amount of dietary magnesium required to prevent the appearance of deficiency symptoms was greater than the amount necessary for optimum growth.
5. The magnesium requirement of pigs weaned at either 3 or 9 weeks of age is between 400 and 500 p.p.m. of the total ration.

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CELLULOSE DIGESTION *IN VITRO* AS A MEASURE OF THE DIGESTIBILITY OF FORAGE CELLULOSE IN RUMINANTS [1, 2]

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PROGRESS in the development of the "artificial rumen" has stimulated interest in the possible application of this technique in the evaluation of forage quality. Pigden and Bell (1955), in a study using 11 forages, obtained a satisfactory regression of percent organic matter digested by sheep on percent "anthrone carbohydrate" fermented *in vitro*. Recently, Belasco *et al.* (1958) utilized cellulose digestion *in vitro* as a criterion in assessing the effect of foliar applications of urea on pasture and forage quality, and Kamstra *et al.* (1958) applied this technique in a study of the factors related to the decreased digestibility of forages, associated with advancing maturity. Such applications imply a close relationship between cellulose digestion *in vitro* and the digestibility of a forage in the animal, although early workers in this field warned that caution should be exercised in applying results obtained by *in vitro* techniques directly to animals. Thus, Burroughs *et al.* (1950) stressed the limitations of the artificial rumen technique with respect to the evaluation of forages, and suggested that it could serve only as a screening device.

The lack of definite information regarding the relationship between digestibility data obtained by *in vitro* and *in vivo* methods prompted the studies reported in the present paper. In carrying out the investigations, an attempt was made to standardize the conditions of the *in vitro* experiments with a view of attaining reproducible results comparable with those obtained in digestion trials.

As the present work neared completion, Barnett (1957) reported a similar study in which good agreement was obtained between cellulose digestion *in vitro* and the digestibility of the crude fiber of silages as measured in sheep trials. Barnett's paper substantiates the general conclusion reached by the present authors, that cellulose digestibility data obtained by the *in vitro* techniques may well prove to be a reliable measure of the digestibility of forage cellulose provided the *in vitro* fermentation are conducted under specified conditions.

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Methods and Materials

Fermentation in vitro. Cellulose digestion *in vitro* was determined by the fermentation technique described by Bentley *et al.* (1955), with slight modifications introduced by Dehority *et al.* (1957) and Johnson *et al.* (1958). For the sake of completeness and clarity the modified technique used in the present study is described in detail.

A steer, fitted with a permanent ruminal fistula and maintained on good quality alfalfa hay, served as the source of rumen microorganisms for all fermentations with certain exceptions detailed where relevant. In the preparation of the inoculum, rumen juice was expelled from a sample of rumen contents (taken prior to the morning feed) by squeezing through cheesecloth in a fruit press. The squeezings thus obtained were used in certain experiments and will be referred to as strained rumen juice (S.R.J.).

Approximately 8 lb. of pressed rumen pulp was mixed with 3 liters of phosphate buffer (1.059 g. Na_2HPO_4; 0.436 g. KH_2PO_4 per liter), and resqueezed in the press. The liquor thus obtained was filtered through cheesecloth and constitutes the phosphate buffer extract (P.B.E.) used as inoculum in some of the experiments.

One liter of P.B.E. was centrifuged in a Sharples supercentrifuge. The sediment retained on the celluloid liner (Bentley *et al.*, 1954) was resuspended, with the aid of a loose-fitting Potter-Elvehjem homogenizer, in 250 ml. phosphate buffer to which approximately 25 mg. cysteine hydrochloride had been added. Only the sediment above a line one inch from the bottom of the liner was used (Dehority *et al.*, 1957). This inoculum which is the improved inoculum described by Johnson *et al.* (1958), was the one used in the majority of the experiments reported in this paper. Where necessary, it will be referred to as resuspended ruminal microorganisms (R.R.M.) to distinguish it from other inoculum with which it was compared.

Fermentation was carried out in round bottom 75 ml. Servall centrifuge tubes. An appropriate weight of forage equivalent to approximately 0.2 gm. cellulose (see preliminary study described under "experimental") and previously ground to pass a 40-mesh screen, was introduced into each tube. Basal medium as indicated in table 1 and distilled water were added to give a total volume of about 50 ml. The tubes were placed in a water bath thermostatically controlled at 39° C., and were stoppered with rubber stoppers fitted with glass inlet and outlet tubes (4 mm. diam.) to permit gassing of the tube contents with CO_2. The inlet tubes were connected to a CO_2 source, the CO_2 was turned on and the contents of the tubes were allowed to equilibrate for about 15 minutes. After equilibration, the contents of each tube was inoculated with 5.0 ml. inoculum (R.R.M.).

In those instances where strained rumen juice or phosphate buffer

extract were used as inocula, no distilled water was added to the tubes after introduction of the basal medium as the volume of inoculum used was increased to 20 ml.

After 2 to 2½ hours fermentation, the pH of the contents of each tube was adjusted to 6.9 by addition of appropriate amounts of a saturated Na_2CO_3 solution. pH adjustment was repeated at intervals throughout the fermentation period which varied from 30 to 72 hours, depending upon the particular experiment. The selection of pH 6.9 in preference to 6.4 to 6.7 recommended by some authors is based on the finding of Cline (1956) that pH 6.9 is optimal for the digestion of purified cellulose by ruminal microorganisms in an inoculum similar to the one used in the present studies.

TABLE 1. BASAL MEDIUM FOR *IN VITRO* FERMENTATION

Component	Volume used per tube
	ml.
Na_2CO_3 (200 mg./ml.)	0.5
10% glucose solution	0.5
Biotin (10 μg./ml.)	1.0
PABA (100 μg./ml.)	0.25
Valeric acid (5 mg./ml.)	3.0
Urea (126 mg./ml.)	0.5
$FeCl_3 \cdot 6H_2O$ (4.4 mg./ml.), $CaCl_2 \cdot 2H_2O$ (5.29 mg./ml.)	0.5
Mineral mixture[a]	10.0

[a] Ten liters of the mineral mixture (pH 6.55) contained the following in grams: NaH_2PO_4, 56.5; NaH_2PO_4, 54.5; KCl, 21.5; NaCL, 21.5; $MgSO_4 \cdot 7H_2O$, 5.82; and Na_2SO_4, 7.5.

Measurement of Cellulose Digestion. At the end of the *in vitro* fermentation the tubes were disconnected and any material adhering to the CO_2 inlet tube was washed back into the centrifuge tube with distilled water. Material adhering to the sides of the centrifuge tubes was loosened by rubbing with a rubber policeman and the insoluble material was spun down in Servall centrifuge (type SP). The supernatant was decanted, care being taken not to disturb the solid material at the bottom of the tube. Residual cellulose was determined by the method of Crampton and Maynard (1938). The acid digestion was carried out in the centrifuge tubes.

The difference between the initial cellulose content of the forage and the residual cellulose after fermentation was taken as a measure of the cellulose digested and was expressed as a percentage of initial cellulose.

Digestion Trials. Four Columbia wethers were used in each trial. The trials were carried out according to the conventional procedure using a 10-day preliminary period and a 10-day collection period. The animals were placed in wooden crates to facilitate urine collection and the feces were collected in sacks attached with harnesses.

Feed, feces and orts samples from the sheep trials were analyzed for cellulose by the method of Crampton and Maynard (1938) and the data were used to calculate the apparent digestion coefficients of the cellulose.

Description of Forages. Seven samples of forage which were grown at this Station [6] and harvested during the 1957 season were used in that part of this study which dealt with a comparison of cellulose digestion *in vivo* and cellulose digestion *in vitro*. The samples consisted of brome grass and orchard grass hays cut at two stages of maturity and three third-crop alfalfa hays representing three stages of maturity. The entire alfalfa plot was subjected to a second cutting on July 26 and the material for use in this study was harvested after 34, 45, and 56 days. Cutting dates and further description of the hays are given in table 2. All the above

TABLE 2. DESCRIPTION OF O.A.E.S. FORAGES

Hay	Date cut (1957)	Description
Brome grass, 1st stage	June 10	Early bloom
Brome grass, 2nd stage	June 26	Seed stage: seed mature
Orchard grass, 1st stage	June 6	Early bloom
Orchard grass, 2nd stage	June 18	Early seed stage
Alfalfa,[a] 1st stage	Aug. 29	34 days regrowth; early bud
Alfalfa,[a] 2nd stage	Sept. 9	45 days regrowth; early bloom-very sparse bloom
Alfalfa,[a] 3rd stage	Sept. 20	56 days regrowth; sparse bloom
Timothy	June 4	Pre-boot stage

[a] Third growth, following a cutting on July 26, 1957.

hays were used in digestion trials as well as in the *in vitro* work.

In addition, cellulose digestibility *in vitro* was determined on samples of hay obtained from Macdonald College (McGill University).[7] These hays had been used in digestion trials and the cellulose digestibility data were provided for comparison with the *in vitro* data obtained locally.

Certain other forages were used in connection with some phases of the work and these will be detailed in connection with the relevant experiments.

Experimental and Results

Substrate Level and Sample Weight. As shown by Kamstra *et al.* (1958), the level of substrate (forage cellulose) influences the amount of digestion

[6] These forages were provided by the Agronomy Department through the cooperation of R. R. Davis and J. L. Parsons.

[7] The authors are indebted to Lewis E. Lloyd and E. W. Crampton for kindly supplying these samples and the relevant digestion trial data.

that takes place in fermentations *in vitro*. In view of the modifications to the *in vitro* technique introduced in the present study, preliminary experiments were conducted in order to establish the optimal sample weight and substrate level required under these conditions.

Two samples of alfalfa hay cut at different stages of maturity during the 1956 season and containing 19.36% and 30.06% cellulose respectively, were used as test materials. The results of these experiments are summarized in table 3.

The data indicate that an initial cellulose level of 0.2 gm. per tube appears most suitable. Selection of this substrate level allows for sample weights ranging from 0.6 to 1.3 gm. representing materials containing from 33% to 15% cellulose (air dry basis).

TABLE 3. *IN VITRO* DIGESTIBILITY OF FORAGE CELLULOSE USING DIFFERENT SUBSTRATE LEVELS AND SAMPLE WEIGHTS

	First stage alfalfa		Second stage alfalfa	
Sample weight	Initial cellulose[a]	Digestibility in 48 hours[b]	Initial cellulose[a]	Digestibility in 48 hours[b]
gm.	gm.	%	gm.	%
0.28	0.05	64.4	0.08	51.5
0.44	.08	66.1	.13	51.7
0.66	.13	66.0	.20	54.2
1.00	.19	66.0	.30	52.1
1.60	.31	64.6	.48	53.4
2.50	.48	60.3	.75	48.8

[a] Corrected to two decimal places.
[b] Average of three experiments; duplicate samples included in each experiment.

Comparison of Cellulose Digestibility as Measured in vitro and in Sheep Trials. The digestibility of the cellulose in the eight O.A.E.S. forages (see table 2) used in sheep digestion trials, was determined using 30-, 48-, 60-, and 72-hour fermentations *in vitro*. Separate samples were used for each of the fermentation periods thus avoiding the necessity of withdrawing aliquots at intervals from any particular tube. Each forage was used in three separate experiments and all fermentations were carried out on duplicate samples in each experiment.

The digestibility *in vitro* of the cellulose in the forages obtained from Macdonald College was determined in experiments similar to those described above, but only 48- and 60-hour fermentation periods were employed.

The means for the digestibility *in vitro* of the cellulose in the various forages were compared with the respective means obtained in the sheep trials using Student's "t-test." The cellulose digestion obtained in a 30-hour fermentation was, in most cases, significantly lower ($P=0.05$) than the apparent digestion coefficient obtained with sheep, while little

difference was observed in the digestion obtained in the 60- and 72-hour fermentation. Only the data obtained with 48- and 60-hour fermentations therefore are presented in table 4.

For nine of the forages the digestibility *in vitro* of cellulose as measured in 48- and 60-hour fermentations did not differ significantly (P=0.05) from the digestion coefficients obtained in sheep trials. These nine forages

TABLE 4. CELLULOSE DIGESTIBILITY AS MEASURED BY THE *IN VITRO* TECHNIQUE AND IN DIGESTION TRIALS

	In vitro digestibility, %				Digestibility in sheep trial, %	
	48 hr.		60 hr.			
Forage[a]	Mean	S.D.	Mean	S.D.	Mean	S.D.
O.A.E.S. forages:						
Brome grass I	53.6	1.7	56.3	1.4	54.7	1.0
Brome grass II	45.0	1.2	46.6	1.5	49.1	2.4
Orchard grass I	68.1	1.8	70.3	1.9	67.0	2.6
Orchard grass II	55.4	1.9	57.6	2.0	59.3	3.7
Timothy (preboot)	59.9	1.9	61.6	1.0	63.9	3.5
Alfalfa I	54.9	1.2	55.2	0.3	57.5	5.1
Alfalfa II	57.4[b]	2.6	56.1[b]	0.6	50.8	1.4
Alfalfa III	52.7	1.2	54.5	1.4	50.9	5.2
Macdonald College forages:						
Timothy	64.4	3.2	68.6	3.5	68.6	5.2
Brome	64.9	1.3	69.1	3.2	65.6	2.8
Alfalfa	54.9[c]	1.5	56.8[c]	1.9	65.7	1.4
Birdsfoot trefoil	58.5[c]	3.7	59.3[c]	3.4	69.2	4.8
Red clover	57.5[c]	1.9	58.9[c]	2.3	63.5	3.8

[a] I, II and III refer to first, second and third stages, respectively.
[b] Statistically significant overestimate of digestibility (P=0.05).
[c] Statistically significant underestimate of digestibility (P=0.05).

included all the grass hays but only two of the legumes, *viz.*, the locally grown first and third stage alfalfa. In the case of the second stage alfalfa, both 48-hour and 60-hour fermentations resulted in a statistically significant overestimate of digestibility. On the other hand, the *in vitro* technique gave estimates of cellulose digestibility that were significantly less than the estimates obtained in the sheep trials, with the three legumes from Macdonald College. Cellulose determinations needed for the Macdonald College digestion trials were carried out there whereas the analyses connected with the *in vitro* experiments were done in our laboratory. Although the same method was used, it is possible that a "between-laboratory" effect may be a factor in explaining the differences between these *in vitro* and *in vivo* results.

In most cases, a 60-hour fermentation gave a slightly better estimate of cellulose digestibility than did a 48-hour fermentation when the sheep trial data are used as the basis of comparison.

Reproducibility of Results Obtained by the in vitro Technique. While good reproducibility of cellulose digestion *in vitro* was obtained from one experiment to the next in a series of three experiments conducted within a period of 10 to 14 days, it appeared desirable to compare results obtained at various times over a period of several months. Data obtained at different times with first and second stage brome and orchard grass proved suitable for this study.

Cellulose digestion was measured in separate series of consecutive experiments run during each of the following periods: August, 1957; October, 1957; December, 1957; March, 1958 and May, 1958. Data were obtained for both 30- and 48-hour fermentations but as they present essentially the same picture, only the 48-hour digestion data are included in table 5. It will be seen that, with the exception of the March 1958 series, good reproducibility of results was obtained from one group of experiments to the next.

TABLE 5. REPRODUCIBILITY OF *IN VITRO* CELLULOSE DIGESTIBILITY MEASURED IN DIFFERENT SERIES OF EXPERIMENTS

		48-hr. *in vitro* cellulose digestibility, %							
		Brome grass				Orchard grass			
		1st stage		2nd stage		1st stage		2nd stage	
Period	No. of experiments[a]	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
August, 1957	2	54.7	0.4	42.0	2.3	65.9	4.0	51.7	3.7
October, 1957	3	53.4	2.9	42.2	3.2	66.9	1.5	53.2	1.3
December, 1957	3	53.5	1.7	45.0	1.2	68.1	1.8	55.4	1.9
March, 1958	3	48.1	2.0	41.0	3.1	62.5	1.5	49.5	3.1
May, 1958	2	56.8	0.7	45.4	2.2	67.7	3.0	55.8	1.0
Mean	13	52.9	3.4	43.1	3.1	66.3	3.1	53.3	3.2

[a] Each fermentation run in duplicate in each experiment.

Using the available data for all forages, estimates were made of the variance of the results obtained in each series of *in vitro* experiments and in the corresponding digestion trials. In general, the variance found in data obtained *in vitro* was less than that found in sheep trial data and in no case was the former significantly greater than the latter ($P=0.1$). In contrast, sheep trial variances were significantly greater than those obtained in experiments *in vitro* in 15 of the 64 comparisons.

In order to get an estimate of the overall variation of the results obtained by the *in vitro* technique, the data obtained in all 48-hour fermentations involving the 13 forages listed in table 4 were pooled and the standard deviation was calculated from the mean square for "within forage" varia-

tion. This standard deviation which is based on 183 observations amounted to 2.78%.

Effect of Feeding Different Forages to the Steer Used as Source of Inoculum. As it seemed likely that microbial population of the rumen might vary according to the type of forage ingested, and that the inoculum obtained from a steer fed one type of forage might not be suitable for estimating the digestibility *in vitro* of the cellulose in a different forage, it was decided to investigate this possibility.

TABLE 6. EFFECT OF FEEDING DIFFERENT FORAGES TO STEER USED AS SOURCE OF INOCULUM

Forage fed as source of inoculum	No. of days on forage	*In vitro* cellulose digestibility, %[a]					
		Brome grass		Alfalfa		Timothy	
		30-hr.	48-hr.	30-hr.	48-hr.	30-hr.	48-hr.
Brome grass	2	32.2	42.0	50.8	56.0	49.6	56.7
	6	31.7	42.5	55.4	59.3	47.9	57.4
	9	33.2	45.5	54.3	58.4	48.7	56.9
	Average	32.4	43.3	53.5	57.9	48.7	57.0
Alfalfa	2	30.7	38.8	48.8	55.2	43.7	54.5
	6	31.7	40.1	53.9	57.1	46.6	58.8
	9	38.9	45.1	57.8	59.2	50.8	58.0
	13	36.7	41.8	57.6	55.1	50.4	57.9
	Average	34.5	41.4	54.5	56.6	47.9	57.3
Timothy	2	33.9	42.9	56.2	57.6	48.9	58.2
	6	34.6	42.7	54.5	59.4	50.4	58.1
	9	34.4	43.4	58.2	60.4	49.2	54.2
	13	34.1	42.2	56.1	57.0	42.6	53.4
	Average	34.2	42.8	56.3	58.6	47.8	56.0

[a] Each figure represents the average results of duplicate fermentations.

The second stage brome grass hay described in table 2, a good quality alfalfa hay, and a timothy hay were fed singly, each for a period of 14 days and in the sequence in which they are listed, to a fistulated steer. Rumen contents were taken on the third, seventh, tenth, and fourteenth day after the steer had been put on a particular hay and an inoculum was prepared for use in the measurement of the 30- and 48-hour digestibility *in vitro* of the cellulose in samples of each of the three forages used as feed for the steer. The digestibility data are presented in table 6. As a result of a laboratory accident, no data are available for the inoculum taken after the steer had been fed brome grass for 13 days.

The data were subjected to an analysis of variance, the digestibility data for each of the three forages used in the fermentations *in vitro* being

treated separately. As expected, there was a significant difference between the cellulose digestion in 30-hour and 48-hour fermentations. The only significant "between feeds" difference was obtained in the case of the 30-hour digestibility of alfalfa. The inoculum prepared when timothy was fed to the steer resulted in cellulose digestion which was significantly greater ($P=0.05$) than that obtained with the inoculum prepared when brome grass was fed.

No such significant differences were obtained in the case of the 48-hour data. On the other hand, the alfalfa and brome digestibility data, but not that for timothy, showed significant differences ($P=0.01$) between the results obtained with inocula taken at different intervals after the steer had been on a particular forage, while the "forage fed" x "days on feed" interaction was significant ($P=0.05$) in the case of the brome and timothy digestibility data. However, careful study of the data failed to reveal any consistent trends. Comparison of the average values (cf. table 6) shows that in general the digestibility of the cellulose *in vitro* in a given forage was the same irrespective of the forage fed to the steer used as a source of inoculum.

Comparison of Inoculum Prepared in Different Ways. The method for preparing the R.R.M. inoculum, used in all the experimental work described above, was developed in this laboratory during studies on the digestibility of purified celluloses and, particularly, on the stimulatory factors known to be present in natural materials. In such studies it was necessary to obtain the ruminal microorganisms as free of extraneous material as possible. Because it could be argued that such precautions are unwarranted when natural forages are used as sources of substrate and because Barnett (1957) had reported the successful use of strained rumen juice as inoculum for the digestion of silage *in vitro,* it was decided to study the possibility of using a simpler inoculum with the technique described herein.

The 48-hour digestibility of the cellulose in first and second stage orchard and brome grass hays (see table 2) was determined using strained rumen juice, the phosphate buffer extract of squeezed rumen contents (preparation P.B.E.) and resuspended ruminal microorganisms (R.R.M.) all derived from the same sample of rumen contents. The four forages and each of the inoculum were used concurrently, each fermentation being carried out in duplicate, in each of three separate experiments.

The average digestion data are given in table 7. Although the digestion obtained with P.B.E. was consistently less than that obtained with R.R.M., the data indicate that little advantage was obtained by centrifuging out the cells from the buffer extracted squeezed rumen contents, and resuspending the microorganisms. Strained rumen juice, however, resulted in digestion lower than that obtained with either of the other two inocula, especially in the case of first stage brome grass and second stage orchard grass hay.

Discussion

The results obtained in this investigation indicate that in the case of the seven grass hays studied, good agreement was obtained between the digestibility of the forage cellulose, as measured in digestion trials, and in the *in vitro* experiments.

In the case of the six legume hays, the results are not as consistent. While cellulose digestion was underestimated by the *in vitro* technique in the case of the alfalfa, red clover and birdsfoot trefoil samples obtained from Macdonald College, there was a tendency to overestimate the digestibility of the cellulose in the second and third stage alfalfa hays produced locally. No explanation can be offered for these discrepancies. However,

TABLE 7. DIGESTIBILITY OF CELLULOSE IN ORCHARD AND BROME GRASS HAYS USING INOCULA PREPARED IN DIFFERENT WAYS

	48-hr. *in vitro* digestibility,[a] %					
	S.R.J.[b]		P.B.E.		R.R.M.	
Forage	Mean	S.D.	Mean	S.D.	Mean	S.D.
Brome, 1st stage	42.8	3.8	46.3	2.9	48.1	2.0
Brome, 2nd stage	38.7	1.5	39.0	1.1	41.0	3.1
Orchard, 1st stage	59.3	1.0	60.3	2.4	62.5	1.5
Orchard, 2nd stage	45.6	2.7	48.1	0.9	49.5	3.1

[a] Average of three experiments with each fermentation run in duplicate in each experiment.
[b] S.R.J., P.B.E. and R.R.M. refer to strained rumen juice, phosphate buffer extract and pressed rumen contents and resuspended ruminal microorganisms, respectively. See text for further details.

it is of interest to recall that Dehority *et al.* (1957) reported evidence which suggested the presence in crude alfalfa extract of a substance which had an inhibitory effect on celluloytic activity when assayed by the *in vitro* fermentation technique. The significance of this observation in relation to the results obtained in the present study has not been investigated, but it would appear that, until more information becomes available, caution should be exercised in evaluating the results of *in vitro* studies with legumes.

Another aspect which may have some bearing on this problem is the fact that the *in vitro* digestion data were obtained with inocula from a steer while the *in vivo* digestion coefficients were obtained with sheep. No study has been made in order to ascertain whether inocula derived from sheep and from cattle will give similar *in vitro* digestibility data.

For the purpose of the present study it was assumed that the cellulose digestion coefficients obtained with the sheep and with cattle are essentially similar. While this assumption is substantiated by the results of various workers (e.g. Jordan and Staples, 1951) there is also a considerable body

of information to the contrary, the paper of Cipolloni *et al.* (1951), which reports a statistical analysis of published data, being of particular significance. The question arises whether the differences between the *in vitro* and *in vivo* digestibility data observed in the case of legumes may be associated with the different animal species used in this investigation. On the other hand, good agreement between cellulose digestibility *in vitro* and cellulose digestibility *in vivo* was obtained with the grass hays although different animal species were involved.

A pertinent question requiring consideration is the duration of the *in vitro* fermentation period which should be employed. The data presented above indicate that while a 30-hour fermentation is too short and a 48-hour fermentation is more satisfactory, a 60-hour fermentation period is to be preferred, especially in the case of more mature forages. While extension of the period to 72-hour has little or no additional advantage as far as the observed cellulose digestion is concerned, the latter period permits a more convenient laboratory work schedule. These findings are in complete agreement with those of Barnett (1957) and are in conformity with the suggestion of Phillipson and Cuthbertson (1956), based on data presented by Balch (1950), that about 80% of a meal of hay disappeared from the rumen of cattle in 48 hours.

Although the *in vitro* technique as used in these investigations offered a reasonably high degree of reproducibility, it would seem advisable, if the technique is to be applied routinely to large numbers of samples, to include at least one standard sample of known cellulose digestibility in each run. This would provide a check on the suitability of each inoculum and on the experimental conditions in each run. The standard sample should consist of a forage rather than a purified cellulose, particularly if a simple medium and inoculum are to be used.

The development of a simple laboratory method for the assessment of the nutritive value of forages has long been a goal of animal nutritionists. In attempting to find a way to circumvent the need for digestion trials, various workers have proposed the use of regression equations for the prediction of the digestibility of a feed from its chemical composition. Raymond (1951) has outlined the major difficulties and limitations of this approach and Schneider *et al.* (1951) have stressed the fact that such equations, based on single feed constitutents, do not take into account the modifying effects of other feed components. The authors are aware of the limitations and shortcomings of the "artificial rumen" or "*in vitro*" technique (Warner, 1956) but they are of the opinion that this laboratory method for estimating the digestibility of cellulose in feedstuffs may be of value to animal nutritionists and plant breeders. This technique might be especially useful where the quantity of plant material available is not sufficient for the determination of digestibility coefficients by conventional methods.

Summary

The digestibility of the cellulose in seven grass hays and six legume hays was determined by an *in vitro* rumen technique using inocula obtained from a steer, and in digestion trials with sheep. No significant difference was observed between results obtained *in vitro* and *in vivo* with grass hays, but, in some of the legume hays, cellulose digestibilities, as measured by the two methods, were significantly different.

Results obtained with the *in vitro* technique showed good reproducibility and, in general, variances were less than those calculated for the corresponding sheep trial data.

In an experiment in which different forages were fed to a steer used as a source of inoculum, the digestibility *in vitro* of the cellulose in these forages was not affected by the type of forage fed the steer.

Little difference was noted when cellulose digestion was measured using strained rumen juice, a phosphate buffer extract of pressed rumen contents or resuspended ruminal microorganisms as inoculum.

Possible use of the *in vitro* technique as a tool in the evaluation of the nutritive value of forages is indicated.

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RELATIVE VALUE OF CAROTENE FROM ALFALFA AND VITAMIN A FROM A DRY CARRIER FED TO LAMBS AND PIGS [1]

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THE relative value on a weight basis of carotene and vitamin A for domestic animals has been known to vary with the level of intake since the classical work of Guilbert *et al.* (1937). In a re-evaluation of the former data, Guilbert *et al.* (1940) reported a carotene to vitamin A ratio of 6:1 at levels of intake to provide for freedom from night blindness, but little or no storage, and a ratio of 10:1 to provide for significant storage. This was confirmed, in general, for fattening lambs by Hoefer and Gallup (1947), for fattening pigs by Braude *et al.* (1941), and for swine during gestation and beginning lactation by Parrish *et al.* (1951). The purpose of this study was to provide information on the relative value of carotene from artificially dehydrated alfalfa and vitamin A from a dry carrier when fed to lambs and pigs, at levels of intake to provide low to medium storage of vitamin A.

Experimental Procedure

Animals and Rations. Twenty-four white-faced lambs of an average initial weight of 45 lb. (range 36 to 70) were obtained from North Central Massachusetts and the University of Connecticut flocks during the period May, 1956 to January, 1957. They were placed in individual pens and fed a basal ration, vitamin A depletion ration, which consisted of a mixture by weight of one-third dried beet pulp and two-thirds of a grain mixture, the latter being composed of the following per ton of mixture: 419.5 lb. ground barley, 500 lb. crimped oats, 500 lb. wheat bran, 150 lb. expeller process linseed oil meal, 150 lb. solvent extracted soybean oil meal, 200 lb. cane molasses, 40 lb. 500-potency B-Y dried fermentation solubles, 20 lb. steamed bone meal, 20 lb. iodized salt and 0. 5 lb. irradiated yeast, Standard Brands, type 36-F, containing 16 million I.U. of vitamin D per lb. This basal ration was fed initially at the rate of 0.034 lb. per lb. of live weight per day and increased every

[1] This study was made in part with funds provided by Wirthmore Feeds Inc., Boston, Mass. The vitamin A supplement was supplied through the courtesy of Dr. M. Hochberg, Nopco Chemical Co., Harrison, N. J., and the B-complex vitamin and antibiotic supplements fed to the pigs by Dr. Ralph Elliott, American Cyanamid Co., Pearl River, N. Y. B. A. Donohue, T. Watts and R. Landeck cared for the animals; Mrs. Mae Miller and Mrs. Elaine Trantum provided computational assistance; and Don Grant aided in obtaining the lambs and pigs. We are indebted to Dr. H. J. Fisher, Conn. Agr. Exp. Sta., New Haven, for the proximate analyses of the feedstuffs. The data are in part from a thesis submitted to the Graduate School of the University of Connecticut by the senior author as partial fulfillment of the requirements for the M.S. degree.

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7-day period thereafter by 0.1 lb. per lamb. After six 7-day periods on the basal ration, or after each lamb's blood plasma vitamin A concentration had decreased to less than 12 mcg. per 100 ml., as determined from bi-weekly blood samples, each lamb was fed in addition to the basal ration one of four levels of carotene, 40, 80, 160, or 320 mcg. per lb. live weight per day, from artificially dehydrated alfalfa leaf meal (containing no additives such as antioxidants or oils) or one of four levels of vitamin A (as alcohol), 8, 16, 24 or 32 mcg. per lb. live weight per day, from a dry carrier (NOPCAY "10", Type IV, Lots V 3581 and V 9177, a mixture of naturally occurring vitamin A esters, 10,000 U.S.P. units per gram). Assignment to level of intake of carotene or of vitamin A was done at random in replicates of eight lambs each. After six consecutive 7-day periods of carotene or vitamin A supplementation, each lamb was slaughtered.

Thirty-two Yorkshire pigs of an average initial weight of 30 lb. (range 13 to 62) were obtained from the University of Connecticut herd during the period of August, 1956 to June 1957. They were placed in individual pens and each was fed a basal ration, vitamin A depletion ration, which consisted of per ton of mixture: 1503.25 lb. white hominy, 420.82 lb. solvent extracted soybean oil meal, 20 lb. steamed bone meal, 20 lb. ground limestone, 20 lb. iodized salt, 0.75 lb. irradiated yeast, Standard Brands, type 36-F, containing 16 million I.U. of vitamin D per lb., 2.0 lb. Lederle's Fortafeed 2–49c containing 2,000 mg. riboflavin, 9,000 mg. niacin, 4,000 mg. pantothenic acid, 10,000 mg. choline chloride, and 60 mg. folic acid per lb., 8.81 lb. Lederle's choline supplement containing 25% by weight of choline choloride, 1.0 lb. Lederle's Profactor B containing 10 mg. vitamin B_{12} per lb., 2.78 lb. Lederle's Aurofac 2-A containing 3.6 g. chlortetracycline per lb., 0.05 lb. $CuSO_4 \cdot 5H_2O$, 0.19 lb. $FeSO_4$ (80% anhydrous), 0.24 lb. $MnSO_4 \cdot H_2O$, 0.10 lb. $ZnCO_3$ (anhydrous), and 0.002 lb. $CoSO_4 \cdot 7H_2O$. This basal ration was fed initially at a rate of 0.04 lb. per lb. of live weight per day and increased every 7-day period thereafter by 0.3 lb. per pig. Some of the smaller pigs also received skim milk for a 3 to 6 week period prior to supplementation with carotene or vitamin A. After six 7-day periods on the depletion ration alone, or after their individual blood plasma vitamin A concentration had decreased to less than 12 mcg. per 100 ml., the pigs in replicates of eight each were randomly assigned to one of the same four levels of intake of carotene or of vitamin A as received by the lambs. After six consecutive 7-day periods on the supplements, the pigs were slaughtered.

Each animal's allowance of artificially dehydrated alfalfa leaf meal was based on the carotene concentration of the leaf meal and the anticipated average live weight of each animal for a particular 7-day period, calculated from the two previous 7-day increases in weight. It was weighed to the nearest 0.1 gm. the day prior to the morning feeding period. The allowance of the vitamin A dry carrier was calculated in the same manner and

weighed out to the nearest 0.01 gm. Sufficient depletion ration was added to equal 0.5 lb. for the lambs and 0.25 lb. for the pigs, except at the higher levels of carotene intake. In the latter case, the depletion ration was increased up to 1.0 lb. to provide a more palatable mixture. The supplement and depletion ration were then thoroughly mixed and fed first each morning. After the supplement mixture had been completely consumed, the remainder of the depletion ration allowance was fed.

TABLE 1. AVERAGE CHEMICAL COMPOSITION OF RATIONS AND SUPPLEMENTS

Items	Percent dry matter	Percent of dry matter					
		Protein	Ether extract	Crude fiber	N.F.E.	Ash	Carotene
							mg./lb.
Basal ration							
Lambs	90.13 ±0.52[a]	15.96 ±0.32	2.49 ±0.10	13.49 ±0.27	61.14 ±0.30	6.90 ±0.26	0.06 ±0.02
Pigs	90.62 ±0.50	21.62 ±0.47	5.57 ±0.20	5.02 ±0.13	60.35 ±0.42	7.45 ±0.18	0.13 ±0.04
Artificially-dehydrated alfalfa leaf meal							
Lambs	89.79 ±0.62	23.15 ±0.27	3.69 ±0.33	18.61 ±0.27	42.97 ±0.47	11.58 ±0.07	76.67 ±3.86
Pigs	89.77 ±0.94	22.94 ±0.21	3.64 ±0.32	18.67 ±0.28	43.16 ±0.50	11.59 ±0.10	77.54 ±4.08
Dry carrier of vitamin A							
Lambs	92.23 ±0.47	40.90 ±0.72	21.95 ±0.90	3.36 ±0.20	28.70 ±0.98	5.09 ±0.14	....
Pigs	91.52 ±0.91	40.72 ±1.17	21.61 ±0.76	3.91 ±0.49	28.54 ±1.26	5.23 ±0.11	....

[a] Standard error of the mean.

Average daily barn temperatures for the lambs ranged between 50 and 89° F. with an average minimum of 59°±6 and an average maximum of 69°±9. Similar values for the pigs ranged between 50 and 84° F. with an average minimum of 62°±6 and an average maximum of 71°±7.

Observations and Analyses. All feeds fed and refused were weighed to the nearest 0.1 lb. Animals were weighed to the nearest pound every 7 days. Venous blood samples were obtained by jugular puncture from the lambs at 14-day periods during the depletion period, on the day prior to addition of the carotene or vitamin A supplements, and at successive 14-day periods during carotene or vitamin A supplementation. Blood samples from the pigs were obtained by truncation of the tail infrequently during vitamin A depletion, the day prior to supplementation, and at successive 21-day periods thereafter. Plasma carotenoids and vitamin A were determined by the method of Kimble (1939). At slaughter, individual livers were obtained and macerated in a Hobart food chopper. Representative

samples were taken and stored at 0° F. until analyzed for carotenoids and vitamin A by a modification of the Gallup-Hoefer procedure (Bunnell *et al.*, 1954).

Samples of the depletion rations, the artificially dehydrated alfalfa leaf meal and the dry carrier of vitamin A, taken at approximately 4-week periods during the course of the experiment, were analyzed for proximate constitutents according to A.O.A.C. (1955) procedures, table 1. Samples of alfalfa leaf meal from each 50 lb. bag, held in storage at 0° F., and of the vitamin A dry carrier, held in storage at 36° F., were taken during the carotene and vitamin A supplementation periods and analyzed for carotene and vitamin A concentrations, respectively. Carotene was determined by the official A.O.A.C. (1950) method, except extraction of the pigments was accomplished by the alternative extraction procedure of method II, first action, in lieu of hot extraction. Vitamin A was determined by a modification of the A.O.A.C. (1950) procedure, for fish liver oils (Anonymous, 1951). The alfalfa leaf meal fed to the lambs contained an average of 169.1 mcg. carotene per gm. with a standard error of 8.5 and that fed to the pigs 170.9±9.0 mcg. per gm. The vitamin A dry carrier contained an average of 2,953.8±65.6 and 2,911.8±69.8 mcg. of vitamin A (as alcohol) per gm., respectively, for the lambs and pigs.

Statistical procedures as outlined by Snedecor (1956) and Cochran and Cox (1957) were used. One pig in replicate III on the 32 mcg. vitamin A intake level died and missing values were estimated by appropriate procedures for a randomized block design.

Results and Discussion

Feed Consumption and Growth. Pigs readily consumed their entire ration allowance with only a few feed refusals occurring among the first replicate during carotene or vitamin A supplementation. Lambs had frequent feed refusals during supplementation. However, the carotene and vitamin A supplements were fed in a small amount of the basal ration(s) prior to feeding the remainder of the ration allowance and no refusals of the supplements were observed. Thus, the amounts of carotene and vitamin A added to the basal ration(s) per unit of live weight were assumed not to be affected. However, it was recognized that the refusal by lambs of a portion of the basal ration probably caused an increase in the variation of the response criteria, since the basal ration contributed a very small amount of carotene, approximately 2 mcg. of carotene per lb. of live weight per day, to each lamb's daily intake. Statistical analysis showed that feed refusals, expressed as the percentage of the total number of supplementation days, were unrelated to the level of either carotene or vitamin A fed.

The average initial live weight the day prior to supplementation with its standard deviation per animal in lb. was for the lambs 70±8, and for the

pigs 63±13. Average daily gains during the six-week supplementation period were, respectively, 0.27±0.13 and 1.17±0.12. Neither source of the vitamin nor level of intake within a source was found to have a significant effect on average daily gains in live weight. This is in agreement

TABLE 2. EFFECT OF GRADED LEVELS OF CAROTENE FROM ARTIFICIALLY DEHYDRATED ALFALFA AND OF VITAMIN A FROM A DRY CARRIER ON PLASMA VITAMIN A CONCENTRATION

Items	Lambs		Pigs	
	Initial value	Av. for supplementation period	Initial value	Av. for supplementation period
	mcg. vitamin A/100 ml.			
Carotene intake (mcg./lb. live wt./d.)				
40	6.4	13.3 (12.7) [a]	8.6	17.0
80	8.8	17.6 (12.8)	9.3	22.4
160	6.7	30.1 (28.9)	8.6	24.7
320	4.1	34.2 (37.7)	7.8	31.4
Vitamin A intake (mcg./lb. live wt./d.)				
8	5.5	15.6 (16.6)	8.9	18.5
16	6.5	28.3 (27.5)	9.0	24.0
24	5.8	40.5 (40.9)	8.6	28.8
32	4.6	35.0 (37.5)	6.8	31.6
Standard deviation of an animal	1.7	6.9 (6.9)	2.1	3.9
Rate of linear change per log (mcg. intake)				
Carotene	...	25.04 (30.29) ±5.88 [b] (±5.88)	...	15.18 ±2.88
Vitamin A	...	37.71 (39.72) ±8.76 (±8.76)	...	21.99 ±4.30

[a] Average for supplementation period, adjusted by covariance for initial plasma vitamin A values.
[b] Standard error of linear rate of change, SB.

with the results reported for pigs by Hentges *et al.* (1952a) and for lambs by Hoefer and Gallup (1947).

Animal health was not a problem in this experiment. In the case of the one pig which died, histological examination showed that death was probably due to several factors: choriomeningitis of the non-purulent type, extensive peritonitis and a possible virus infection.

Plasma and Liver Carotenoid and Vitamin A Concentrations. As is characteristic of lambs and pigs, no plasma carotenoids were observed.

Plasma vitamin A increased at diminishing rates with increasing carotene or vitamin A intake, table 2—columns 3 and 5. As was indicated by Almquist (1953) and more recently with calves by Rousseau *et al.* (1956) responses of plasma vitamin A on the logarithm (base 10) of carotene or vitamin A intake resulted in essentially linear relationships. The regressions of the linear change of plasma vitamin A on the logarithm of intake accounted for the following percentages of the total variability about their respective intakes: with carotene supplementation, for lambs 89.98 (adjusted values) and for pigs 96.92; and with vitamin A supplementation 89.40 and 99.15, respectively. There was slight indication of curvature in the regression of plasma vitamin A on log vitamin A intake for the lambs; however, the sums of squares attributable to the introduction of the square of log intake were of relatively small magnitude and not statistically significant.

Apparent liver carotenoids increased with increasing carotene intake for both lambs and pigs, table 3. Liver carotenoids decreased with increasing vitamin A intake for the lambs and an inappreciable change was observed for the pigs. Liver vitamin A increased at essentially constant rates with increases in carotene or vitamin A intake. The variation in both liver carotenoids and liver vitamin A concentrations was found to increase with increasing levels of carotene or vitamin A intake. The transformation of these criteria to their respective logarithms (base 10) minimized these variations and resulted in variances which did not differ significantly among intake groups as indicated by Bartlett's test of homogeneity of variance. This was comparable to the results obtained with liver vitamin A data of calves (Rousseau *et al.*, 1956). An essentially linear relationship was obtained for the response of log liver vitamin A or log liver carotenoids+1 on log carotene intake or on log vitamin A intake. The regressions of the linear change of log liver concentrations on log intake accounted for the following percentages of total variability of log liver vitamin A about their respective intakes: with carotene supplementation for lambs 95.28 and for pigs 99.81 and with vitamin A supplementation, 97.46 and 98.90, respectively. Similar values for log liver carotenoids+1 were: with carotene supplementation, for lambs 86.24 and for pigs 81.75; with vitamin A supplementation, 61.53 and 42.72, respectively.

From these linear relationships of plasma or log liver vitamin A on log intake for both lambs and pigs, the data indicated that per unit of intake, vitamin A gave a greater rate of change than a corresponding increase in carotene intake. These results confirm those cited in the introduction of this paper.

Relative Value of Carotene and Vitamin A. The amounts of vitamin A from a dry carrier necessary to obtain an equivalent response to the 40, 80, 160 or 320 mcg. carotene intake from alfalfa based on plasma or log liver vitamin A concentrations were derived from the regressions of the respective response criteria on log intakes of carotene or vitamin A.

TABLE 3. EFFECT OF GRADED LEVELS OF CAROTENE FROM ARTIFICIALLY DEHYDRATED ALFALFA AND OF VITAMIN A FROM A DRY CARRIER ON LIVER CAROTENOID AND VITAMIN A CONCENTRATIONS

Items	Lambs				Pigs			
	Carotenoids		Vitamin A		Carotenoids		Vitamin A	
	mcg. per 100 gm.	Log (mcg. per 100 gm. +1)	mcg. per 100 gm.	Log (mcg. per 100 gm.)	mcg. per 100 gm.	Log (mcg. per 100 gm. +1)	mcg. per 100 gm.	Log (mcg. per 100 gm.)
Carotene intake (mcg./lb. live wt./d.)								
40	18	1.27	120	2.02 (1.96) [a]	11	1.04	379	2.48
80	28	1.44	320	2.46 (2.26)	13	1.08	529	2.74
160	28	1.44	467	2.60 (2.56)	15	1.16	965	2.97
320	35	1.55	1377	3.04 (3.18)	39	1.50	1699	3.18
Vitamin A intake (mcg./lb. live wt./d.)								
8	5	0.59	132	2.03 (2.07)	2	0.30	316	2.35
16	6	0.65	764	2.86 (2.83)	2	0.36	673	2.69
24	4	0.44	1201	3.05 (3.07)	2	0.30	1183	2.96
32	3	0.38	1515	3.14 (3.24)	2	0.42	1659	3.19
Standard deviation of an animal	..	0.36	...	0.23 (0.22)	..	0.18	...	0.20
Rate of linear change per log (mcg. intake)								
Carotene	..	0.27 ±0.31 [b]	...	1.07 (1.28) ±0.20 (±0.20)	..	0.49 ±0.13	...	0.77 ±0.14
Vitamin A	..	−0.38 ±0.46	...	1.87 (1.95) ±0.29 (±0.29)	..	0.14 ±0.19	...	1.38 ±0.22

[a] Liver vitamin A values adjusted by covariance for plasma vitamin A values determined the day prior to supplementation.
[b] Standard error of linear rate of change, s_b.

For example, lamb plasma vitamin A in mcg./100 ml. (unadjusted), y, on log carotene intake in mcg./lb. live weight/day, x_1, resulted in the linear function 1.) of $y=-27.63+25.04x_1$, and on log vitamin A intake, x_2, in the linear function 2.) of $y=-17.22+37.71x_2$. Setting y in function 1.)

TABLE 4. RELATIVE VALUE OF CAROTENE FROM ARTIFICIALLY DEHYDRATED ALFALFA AND VITAMIN A FROM A DRY CARRIER

Amount of carotene from alfalfa fed	Amount of vitamin A from a dry carrier necessary to obtain equivalent responses to carotene	Ratio of carotene to vitamin A
mcg./lb. live wt./day		mcg. carotene/ mcg. vit. A
Based on plasma vitamin A		
Calves[a]		
40	9.9	4.0
80	12.5	6.4
160	16.6	9.6
320	21.9	14.6
Lambs		
40	6.1 (5.2)[b]	6.6 (7.7)[b]
80	9.7 (8.8)	8.2 (9.1)
160	15.4 (14.9)	10.4 (10.7)
320	24.4 (25.2)	13.1 (12.7)
Pigs		
40	7.1	5.6
80	11.5	7.0
160	18.5	8.6
320	29.9	10.7
Based on log liver vitamin A		
Calves[a]		
40	7.6	5.3
80	10.2	7.8
160	13.7	11.7
320	18.4	17.4
Lambs		
40	7.3 (6.2)[b]	5.5 (6.4)[b]
80	10.8 (9.8)	7.4 (8.2)
160	16.0 (15.5)	10.0 (10.3)
320	23.8 (24.4)	13.4 (13.1)
Pigs		
40	10.7	3.7
80	15.7	5.1
160	23.1	6.9
320	34.0	9.4

[a] Calculated from the data of Rousseau *et al.* (1956).

[b] Values calculated from the plasma and liver vitamin A concentrations adjusted by covariance for the plasma vitamin A values determined the day prior to vitamin supplementation.

to equal y in function 2.) and solving for x_2, gave equation 3.) $x_2 = -0.28 + 0.66x_1$. This linear function of log vitamin A intake on log carotene intake was then used to determine quantities of vitamin A, x_2, by substitution of appropriate intake quantities of carotene, x_1.

Carotene to vitamin A (alcohol) equivalencies on a weight basis from the data in this experiment as well as those calculated from the data of Rousseau *et al.* (1956) for calves are contained in table 4. Calves, lambs and pigs appeared to be somewhat equal in the conversion of carotene to vitamin A at the two lower levels of carotene intake, but at the two higher levels, the pigs appeared to be more efficient than either calves or lambs and the lambs more efficient than calves. However, these apparent differences among species need to be interpreted with caution, since, they represent single experiments with limited numbers of animals per experiment. Also, they apply only to the particular source of carotene or vitamin A, for it has been shown that the availability is dependent upon this factor (Hoefer and Gallup, 1947; Hentges *et al.*, 1952b). The data provide information over ranges of carotene and vitamin A intakes which are likely to be used in practice and point out the need, as done by Guilbert *et al.* (1940), to express carotene or vitamin A on a weight basis rather than on an I.U. or U.S.P. unit basis.

Summary

Graded levels, 40, 80, 160 and 320 mcg. of carotene from artificially dehydrated alfalfa leaf meal or, 8, 16, 24 and 32 mcg. of vitamin A from a dry carrier per lb. of live weight per day were added to vitamin A depletion rations of 24 lambs and 32 pigs, partially depleted of their vitamin A stores, for a period of 6 weeks duration. Daily gains were not affected. Plasma vitamin A concentrations increased at diminishing rates and liver vitamin A concentrations increased at essentially constant rates with increasing intakes of carotene or vitamin A. Liver vitamin A was transformed to logarithms to minimize variances and along with plasma vitamin A resulted in essentially linear regressions on logarithms of intake. A greater rate of response with increasing vitamin A intake than with increasing carotene intake was observed. The relative values of carotene to vitamin A were determined from these linear regressions. Based on plasma vitamin A, the following carotene to vitamin A (alcohol) ratios on a weight basis were found: At the 40 mcg. carotene intake level, for the lambs 8:1 and for the pigs 6:1; at 80 mcg., respectively, 9:1 and 7:1; at 160 mcg., 11:1 and 9:1; and at 320 mcg., 13:1 and 11:1. Similar values based on log liver vitamin A were: At the 40 mcg. carotene intake level, 6:1 and 4:1; at 80 mcg., 8:1 and 5:1; at 160 mcg., 10:1 and 7:1; and at 320 mcg., 13:1 and 9:1.

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PRENATAL REPRODUCTIVE PERFORMANCE IN GILTS FED LOW LEVELS OF STILBESTROL IN A LEGUME-FREE AND AN ALFALFA RATION [1, 2]

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IN previous studies gilts fed a ration containing 18% sun-cured alfalfa hay farrowed more live pigs, and a greater percentage of their pigs lived to weaning age than gilts fed a fortified legume-free ration (Teague, 1955). It was further observed that the feeding of alfalfa resulted in an increase in the average number of eggs ovulated (corpora lutea) but did not appear to influence prenatal survival.

Several of the fresh, cured or ensiled forages which are commonly included in feeds for domestic animals have been shown to contain measureable quantities of estrogenic materials (Pieterse and Andrews, 1956 and Stob and Andrews, 1957). Cheng *et al.* (1953) reported that second cutting alfalfa contains estrogenic activity equivalent to 1.61 mcg. of stilbestrol per lb. These levels of estrogenic activity are comparable to those shown to be physiologically active in the female reproductive system of several species (Robinson and Moore, 1956; Byrnes and Meyer, 1951; Correa Freire, 1955; Paesi, 1952).

The experiments reported were conducted to determine whether stilbestrol additions to the ration comparable to the estrogenic activity of alfalfa influence reproductive performance in swine.

Experimental Procedure

A total of 189 Duroc gilts selected from the Experiment Station herd were used in experiments conducted during five successive breeding seasons; beginning in the fall of 1955 and continuing through the 1957 fall season. Allotment in each experiment was according to weight and relationship. Genetic variability was reduced by distributing full sibs and half sibs as uniformly as possible in all lots. The average initial age of gilts by lots were in a range from 208 to 254 days in the five experiments. Average beginning weights by lots were from 244 to 287 lb. The design of experiments and ration treatments are shown in table 1.

The composition of the 18% alfalfa and legume-free rations are shown in table 2. Supplementation of the legume-free ration with vitamins and

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[2] Published with the approval of the Associate Director, Ohio Agricultural Experiment Station as Journal Article No. 55–58.

TABLE 1. EXPERIMENTAL DESIGN

Ration	Stilbestrol added per lb. of ration	Number of gilts per lot					
		Fall 1955	Spring 1956	Fall 1956	Spring 1957	Fall 1957	All experiments
Legume-free	None	8	8	8	13	8	45
Legume-free	0.29 mcg.	8	8	8	..	16	40
Legume-free	0.58 mcg.	8	8	..	13	10	39
Legume-free	3.00 mcg.	..	..	..	13	20	33
18% alfalfa	None	8	..	8	..	..	16
18% alfalfa	0.29 mcg.	8	..	8	..	..	16

TABLE 2. COMPOSITION OF RATIONS

Ingredient	Legume-free	18% alfalfa
	%	%
Ground shelled corn	....	53.2
Ground ear corn	70.3	....
Ground oats	14.2	15.0
Meat and bone scraps (50%)	5.8	5.2
Soybean meal[a] (44%)	7.2	6.6
Ground alfalfa (sun-cured)	....	18.0
Mineral mixture no. 3[b]	1.5	1.0
Irrad. yeast, 9f-corn premix[c]	1.0	1.0

Vitamin supplements and Mn for each 100 lb. of legume-free ration:

Vitamin A supplement[d]	34.2 gm.
Vitamin D, irradiated yeast 9f	2.0 gm.
Riboflavin	225.0 mg.
Niacin	625.0 mg.
Pantothenic acid	295.0 mg.
25% dry choline supplement[e]	66.4 gm.
Folic acid supplement (Parvo)[f]	4.8 gm.
Pyridoxine	170.0 mg.
Thiamin	50.0 mg.
Biotin	3.0 mg.
Wheat germ oil[g]	11.35 gm.
Manganese sulfate	1.20 gm.

[a] Includes 1 lb. added as the vitamin premix to the legume-free diet.
[b] Ground limestone, 38.4; special steamed bone meal, 38.4; trace mineralized salt, 19.2; and ferrous sulfate, 4.0%.
[c] Contains 1 lb. of irradiated yeast (9f) in 100 lb. of ground shelled corn.
[d] Pfizer's Vitamin A Supplement 10, a synthetic vitamin A (acetate) feed supplement containing 10,000 I.U. per gm.
[e] Dry mixture of choline chloride containing 25% choline chloride and 75% corn distillers dried grains (Merck).
[f] A folic acid supplement containing not less than 3% folic acid (Lederle).
[g] Rex wheat germ oil (Vio Bin Corporation).

manganese supplied known nutritional constituents of alfalfa. The amounts of B-vitamins and vitamins A and D were calculated to be equal to twice that which would be supplied by 18% alfalfa. Ground ear corn was employed in the legume-free ration to more nearly equalize the fiber content of the two rations. For the alfalfa-containing rations, good quality second cutting alfalfa hay was selected. To improve uniformity of ingredient composition, corn, oats and alfalfa from the same sources, in quantities sufficient to complete the experiment, were secured at the beginning of each study.

Equal amounts of the rations were hand fed to the gilts twice daily. The amount fed was adjusted to promote an average daily gain of approximately one lb. per gilt.

Stilbestrol was incorporated into the rations by first premixing weighed quantities of corn oil containing 1 to 10 mcg. of stilbestrol per gram of oil in 20 lb. of soybean oil meal for each 1000 lb. of ration. The stilbestrol premix was then added to the other ration components and mixed in a 1-ton vertical auger-type mixer. Stilbestrol-containing rations were mixed after the control rations and the mixer was cleaned thoroughly between each batch of feed. Each ration was stored in the same bags throughout the experiment to avoid stilbestrol contamination.

Breeding was started after an initial feeding period of 29 to 30 days. The breed of boars, whether Duroc, Yorkshire, or crossbred, was the same within each of the experiments conducted. The use of individual boars was uniformly distributed among the lots in each study. Gilts were bred, a single service in all cases, on the first day they demonstrated estrus. Those gilts which failed to conceive at the first mating were bred a second time. Any gilts not settled after the second mating were slaughtered and examined for abnormalities of the reproductive system.

Twenty-five to twenty-seven days following breeding, the females were slaughtered. The uteri and ovaries were returned to the laboratory for examination. In the fall, 1956 experiment this procedure was delayed until 55 to 57 days following breeding. Observations on the reproductive organs included number of corpora lutea and number of live, dead and resorbing embryos. Viability of embryos was based upon their size and appearance.

Results

The average weight gains were similar for gilts fed equal quantities of the legume-free and 18% alfalfa rations, with or without added stilbestrol.

Estrual behavior did not appear to be altered by any of the ration treatments. The intensity and length of estrus as well as boar acceptance was similar in all groups. No marked differences in length of the estrous cycle were observed. Failure to conceive at first or second breeding was not associated with the ration fed or stilbestrol additions. In most cases,

TABLE 3. PRENATAL OBSERVATIONS ON GILTS FED LOW LEVELS OF STILBESTROL IN A LEGUME-FREE RATION OR AN 18% ALFALFA RATION

Experiment	Fall 1955	Spring 1956	Fall 1956	Spring 1957	Fall 1957	All experiments
Days of gestation	25–27	25–27	55–57	25–27	25–27	
Legume-free ration:						
Number of gilts[a]	8	8	8	12	6	42
Av. number corpora lutea	14.62	18.12	13.50	14.50	13.67	14.52
Av. number live embryos	10.38	13.50	11.12	11.75	11.83	11.71
Percent corpora lutea represented as live embryos	70.94	83.72	82.41	81.03	87.58	80.66
Legume-free ration+0.29 mcg. stilbestrol per lb.:						
Number of gilts	7	7	7	.....	15	36
Av. number corpora lutea	12.57	14.43	13.86	.....	13.67	13.64
Av. number live embryos	7.29	12.14	11.00	.....	11.73	10.80
Percent corpora lutea represented as live embryos	57.95	84.16	79.38	.....	85.85	79.23
Legume-free ration+0.58 mcg. stilbestrol per lb.:						
Number of gilts	8	8	.....	11	10	37
Av. number corpora lutea	13.62	14.10	.....	13.73	12.90	13.55
Av. number live embryos	7.62	10.88	.....	10.64	10.90	10.11
Percent corpora lutea represented as live embryos	55.96	76.99	.....	77.48	84.50	74.50
Legume-free ration+3.0 mcg. stilbestrol per lb.:						
Number of gilts	.....	.....	.....	12	19	31
Av. number corpora lutea	.....	.....	.....	14.00	13.35	13.59
Av. number live embryos	.....	.....	.....	12.83	11.11	11.77
Percent corpora lutea represented as live embryos	.....	.....	.....	91.66	79.03	83.91
Eighteen percent alfalfa ration:						
Number of gilts	8	.....	8	.....	.....	16
Av. number corpora lutea	14.12	.....	13.12	.....	.....	13.62
Av. number live embryos	11.00	.....	11.00	.....	.....	11.00
Percent corpora lutea represented as live embryos	77.88	.....	83.80	.....	.....	80.73
Eighteen percent alfalfa ration+0.29 mcg. stilbestrol per lb.:						
Number of gilts	8	.....	8	.....	.....	16
Av. number corpora lutea	14.38	.....	12.87	.....	.....	13.62
Av. number live embryos	11.00	.....	10.75	.....	.....	10.88
Percent corpora lutea represented as live embryos	76.52	.....	83.50	.....	.....	79.82

[a] Number of gilts in each lot that settled and possessed embryos when slaughtered.

failure to conceive after breeding the second time could be related to an anatomical abnormality of the reproductive organs.

Vulval swelling, in the absence of estrus, appeared to be associated with the feeding of stilbestrol in the legume-free ration. This was most apparent in the fall, 1955 study and was demonstrated to a lesser extent in the succeeding experiments. The subjective nature of this observation proved difficult to evaluate quantitatively. The vulval swelling in gilts fed the alfalfa-containing ration or the alfalfa-containing ration with stilbestrol did not differ noticeably from that of gilts fed the legume-free ration without stilbestrol added.

The prenatal reproductive performance for all experiments is summarized in table 3. Consideration of the means for each of the treatment groups suggests that ovulation rate (corpora lutea), number of live embryos and percent corpora lutea represented as live embryos were reduced when stilbestrol was added to the legume-free ration. No influence on performance was observed when stilbestrol was added to the 18% alfalfa ration.

A multiple regression analysis of the data obtained from gilts fed the legume-free rations revealed that both ovulation rate and number of live embryos was positively correlated with weight of the gilt. This correlation was statistically significant at the 5% level of probability. Within the age range used in these experiments there was no association of age of gilt to ovulation rate or number of embryos. This same technique of analysis also showed that the general level of performance, regardless of treatment, varied considerably from one experiment to another but the variation was not related to a particular season, i.e., spring *vs.* fall.

The number of corpora lutea and live embryos and the percent corpora lutea represented as live embryos were corrected to adjust for the variation between experiments by using a multiple regression technique. Corrections were also made for the association of weight with corpora lutea and live embryos. The corrected data for prenatal observations in all experiments were then combined and are summarized in table 4.

The means for corpora lutea, live embryos and percent corpora lutea represented as live embryos were tested for equality by the analysis of variance. The summaries of these analyses are shown in table 5.

Ovulation rate was lower when any of the three levels of stilbestrol were included in the legume-free ration. The reduction in ovulation rate of 0.75, 1.11 and 1.06 ova for the 0.29, 0.58 and 3.0 mcg. levels of stilbestrol, respectively, was significant at the 10% level of probability. Although the data suggests a negative regression of ovulation rate on stilbestrol treatment, the number of animals used in these experiments was not adequate to demonstrate this statistically.

The average number of live embryos was also lower when stilbestrol additions were made. This was in addition to the reduction that would

TABLE 4. PRENATAL OBSERVATIONS ON GILTS FED LOW LEVELS OF STILBESTROL IN A LEGUME-FREE RATION CORRECTED FOR VARIATION DUE TO SEASON AND WEIGHT OF GILT

Treatment	No. of gilts	Corpora lutea Av.	Corpora lutea S.D.	Live embryos Av.	Live embryos S.D.	Corpora lutea represented as live embryos, % Av.	Corpora lutea represented as live embryos, % S.D.
Legume-free ration	42	14.35	±2.49	11.67	±3.01	85.8	±5.6
Legume-free ration+0.29 mcg. stilbestrol per lb.	36	13.60	±2.06	10.14	±2.86	80.7	±6.3
Legume-free ration+0.58 mcg. stilbestrol per lb.	37	13.24	±1.53	9.69	±2.67	76.1	±4.7
Legume-free ration+3.0 mcg. stilbestrol per lb.	31	13.29	±1.77	10.08	±3.23	84.0	±6.8

be reflected by the lower ovulation rate associated with the stilbestrol treatments. The mean differences for live embryos were significant at the 5% level of probability and the within treatment variations, as indicated by standard deviations, were similar. In the spring, 1957 experiment the average number of live embryos observed in gilts fed 3.0 mcg. of stilbestrol per lb. of ration exceeded that of the control group which received no stilbestrol by 1.08. Since ovulation rate for these gilts was lower, a high proportion of the ova shed developed into live embryos. The 91.7% of

TABLE 5. ANALYSIS OF VARIANCE SUMMARIES FOR CORPORA LUTEA, LIVE EMBRYOS AND PERCENT CORPORA LUTEA REPRESENTED AS LIVE EMBRYOS OBSERVED IN GILTS FED LEGUME-FREE RATIONS

Source	Total SS	df	MS	F
Corpora lutea				
Total	1316.39	145	.....	....
Treatment	91.41	3	30.47	2.58*
Error	1224.97	142	8.63	....
Live embryos				
Total	599.15	145	.....	....
Treatment	31.05	3	10.35	3.53*
Error	586.09	142	4.00	....
Percent corpora lutea represented as live embryos				
Total	28920.10	145	.....	....
Treatment	1421.08	3	473.69	2.45*
Error	27499.01	142	193.66	....

* Significant at 0.05 level of probability.
* Significant at 0.10 level of probability.

corpora lutea represented as live embryos in this group was the highest observed in all the experiments and was 10.6% higher than that for the control animals. In the fall, 1957 study, the high rate of embryo survival was not associated with this same treatment and in this case was 8.6% lower than the controls. The corrected and combined data for this measurement gave a value approximately equal to the control group.

The percent of corpora lutea represented as live embryos was consistently reduced when 0.29 and 0.58 mcg. of stilbestrol per lb. was added to the legume-free ration. When the data were corrected this reduction was approximately 5% on the 0.29 mcg. level and approximately 10% on the 0.58 mcg. level.

In the fall, 1955 and fall, 1956 experiments inclusion of alfalfa in the ration was not associated with differences in performance when compared with that obtained on the legume-free ration. When stilbestrol was added to the 18% alfalfa ration at the rate of 0.29 mcg. per lb. the performance of gilts was similar to that of those which received the alfalfa-containing ration without stilbestrol.

Discussion

Under the experimental conditions employed, levels of 0.29 to 3.0 mcg. of stilbestrol per lb. of legume-free ration appear to have been physiologically active in female swine. Vulval swelling that occurred when estrus was not demonstrated was one indication of this, although this effect was not consistent or readily quantified. A similar response was observed by Koen and Smith (1945) when swine were fed moldy corn and by Shoop and Klette (1955) when swine were fed moldy rye. These authors suggested that the vulval stimulation was related to estrogenic activity in the moldy feeds.

The daily stilbestrol intake of each gilt was approximately 1.8, 3.2 and 18 mcg., respectively, for the three levels used. Bioassays of feed samples in two of the experiments indicated that the additions of stilbestrol were uniformly distributed and that the estrogenic activity was effectively increased. Absorption of these low levels of orally administered stilbestrol is substantiated by the work of Hanahan *et al.* (1953). Using rats the pathway for absorption of C^{14}-diethylstilbestrol appeared to be via the portal rather than the lymph system regardless of the type of carrier used.

The reduction of ovulation rate associated with the addition of stilbestrol to the legume-free ration further suggests that these levels were physiologically active. Although the mechanism involved was not indicated from observations in these experiments, evidence in other species suggests that the levels employed could influence the ovarian-hypophyseal axis. Byrnes and Meyer (1951) demonstrated that estrogen doses from 0.015 to 0.175 mcg. affected FSH and LH secretion in the immature

and adolescent female rat. Paesi (1952) also postulated that the release of FSH and LH was influenced by dosages of 0.01 to 0.05 mcg. estradiol which were shown to produce an ovarian response in immature rats.

The smaller number of live embryos and lower embryo survival which were associated with the low levels of oral stilbestrol was similar to the effects which have been noted from the injection of estrogen in other species. Correa Freire (1955) observed that complete or partial abortion resulted when rats received 0.1 to 5.0 mcg. of estradiol early in gestation. Kelly (1931) found that 0.3 to 3.6 mcg. estrin prevented conception in the guinea pig while D'Amour *et al.* (1933) showed that 0.45 mcg. prevented conception in the rabbit. The point at which stilbestrol feeding influenced the number of live embryos, whether at fertilization or as an influence on uterine environment early in gestation, was not indicated by the data available. Since progesterone-estrogen ratios are critical in both instances and estrogen can neutralize the effects of progesterone in ratios of 1 to 1000 and more (Courrier, 1950) one or both reproductive processes may have been influenced.

Continued feeding of stilbestrol until midway through gestation did not further decrease the number of live young. This suggests that the effect of reducing litter size occurred prior to the 25th day of gestation.

The reproductive performance of gilts fed the alfalfa-containing ration was not higher than the performance of those fed the legume-free rations as noted in earlier studies by Teague (1955). This apparent improvement in performance on the legume-free ration cannot be explained.

Summary

In five dry-lot experiments, gilts were fed legume-free or 18% alfalfa, breeding-gestation rations containing levels of 0.29, 0.58 and 3.0 mcg. of stilbestrol per lb. A feeding period of 30 days preceded breeding. The gilts were slaughtered during gestation and observations made on the number of corpora lutea and live young.

Lower ovulation rates were associated with the inclusion of the three levels of stilbestrol in the legume-free ration. The number of live young and percent corpora lutea represented as live embryos was consistently reduced when 0.29 and 0.58 mcg. of stilbestrol per lb. were included in the legume-free ration. The average number of live embryos and percent corpora lutea represented as live embryos observed in gilts fed 3.0 mcg. of stilbestrol per lb. of legume-free ration exceeded that of the control group in one experiment but were lower in the succeeding experiment. The addition of stilbestrol to the 18% alfalfa ration did not influence reproductive performance as measured.

Corpora lutea and live young were positively correlated with weight of the gilts regardless of age.

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PROGESTERONE CONTENT OF BOVINE REPRODUCTIVE ORGANS AND BLOOD DURING PREGNANCY [1]

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PROGESTERONE is associated with several different aspects of bovine reproductive physiology. For example, Melampy *et al.* (1957) have demonstrated that this hormone can induce estrous behavior in the estrogen-conditioned ovariectomized cow and Turner *et al.* (1956) have reported that it functions in the induction of lobule-alveolar mammary gland growth. In addition, progesterone is necessary for the maintenance of early pregnancy in the cow, according to McDonald *et al.* (1952).

The study reported here deals with the progesterone content of reproductive tissues and fluids, adrenal gland and blood of the cow during pregnancy.

Experimental Methods

Reproductive tracts were obtained from Hereford and Angus cows in different stages of gestation. The stage of pregancy of each cow was evaluated by the crown-rump length of the fetus according to Stoss (1944). The crown-rump length was measured from the fontanel to the tuber ischium. Progesterone determinations were made on corpora lutea, residual ovarian tissue, placentae, allantoic and amniotic fluids and left adrenal glands. Tissue samples were frozen at time of collection and stored for analysis. Blood samples taken from Holstein cows in different stages of pregnancy were also analyzed for progesterone. The chemical procedure used for the progesterone determination is described in detail by Rakes *et al.* (1958). Analysis of variance was used in the statistical evaluation of data.

Pooled extracts eluted from chromatograms were further purified by counter-current distribution (Pearlman, 1954) for infra-red analysis and qualitative evaluation using the Zimmerman reaction (Savard, 1953). Infra-red analysis was carried out using the Nujol mull technique.

Results

Data on the concentration of progesterone (mcg. per gm.), on a fresh-weight basis, in organs and reproductive fluids are presented in table 1.

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TABLE 1. CONCENTRATION OF PROGESTERONE IN ORGANS AND FLUIDS DURING PREGNANCY OF THE COW

Stage	Duration of pregnancy in days	No. of animals	Micrograms progesterone per gram (fresh-weight basis) [a]									
			Corpus luteum		Residual ovarian tissue		Placenta		Allantoic fluid		Amniotic fluid		Left adrenal gland	
1	10–49	9	2.3	0.7 [a]	1.1	0.5	0.08	0.04	0.06	0.01	0.02 [b]	....	...	....
2	50–89	3	3.6	1.5	1.0	0.4	0.08	0.03	0.08	0.01	0.01	0.003	1.5 [b]	....
3	90–129	13	5.0	1.2	2.7	0.6	0.16	0.09	0.07	0.01	0.04	0.01	1.7	0.04
4	130–169	14	3.8	0.9	1.6	0.5	0.20	0.06	0.07	0.01	0.04	0.01	1.5	0.09
5	170–209	9	3.9	0.7	1.9	0.5	0.20	0.07	0.06	0.01	0.01	0.002	2.1	0.09
6	210–249	9	1.5	0.5	0.7	0.1	0.05	0.02	0.03	0.01	0.02	0.01	0.4 [b]	....
7	250–280	2	1.1	0.3	0.8	0.5	0.03	0.01	0.04	0.01	0.02 [b]	....	0.7 [b]	....

[a] Means and standard errors, ±.
[b] One sample.

Results dealing with the total progesterone in mcg. per organ and total quantity of reproductive fluids are summarized in table 2. The data obtained from the analysis of tissues and fluids from each cow are grouped to represent seven stages during pregnancy of the cow. Each stage represents a period of 40 days, with the exception of stage 7 (30 days) e.g., 1=10–49 days; 2=50–89 days, etc. Data on the mcg. of progesterone per 100 ml. of whole blood collected at different stages of gestation are presented in table 3.

Pooled extracts from corpora lutea, residual ovarian tissue, placenta, adrenal, allantoic and amniotic fluids and blood gave positive results with a Zimmerman reaction. The infra-red absorption curves obtained on extracts of corpora lutea, residual ovarian tissue, placentae, and blood were characteristic for a carbon-20 carbonyl group as shown by the peak

TABLE 2. MICROGRAMS OF TOTAL PROGESTERONE IN ORGANS AND FLUIDS DURING PREGNANCY OF THE COW [a]

Stage	Duration of pregnancy in days	No. of animals	Corpus luteum	Residual ovarian tissue	Placenta	Allantoic fluid	Amniotic fluid	Left adrenal gland
1	10–49	9	10 4	6 2	1 1	39 9	2 [b]	
2	50–89	3	19 9	8 1	5 2	58 12	2 1	17 [b]
3	90–129	13	23 5	17 3	71 10	138 38	36 5	15 4
4	130–169	14	17 4	16 5	92 50	253 60	253 54	16 7
5	170–209	9	16 5	13 5	403 145	347 60	320 79	20 1
6	210–249	9	8 2	7 2	100 30	277 39	331 78	16 [b]
7	250–280	2	7 1	8 3	115 30	314 46	73 [b]	9 [b]

[a] Means and standard errors, ±.
[b] One sample.

at 1707 cm.$^{-1}$ and a△–4, 3-keto group as shown by a peak at 1670 cm.$^{-1}$. The results obtained with the Zimmerman reaction and infra-red analysis indicate the presence of progesterone in the extracts evaluated.

Discussion

As shown in table 1, luteal tissue contained the highest concentration of progesterone and residual ovarian tissue the next highest. The progesterone present in residual ovarian tissue may have been produced by scattered luteal cells or may have diffused from the active corpus luteum of pregnancy. The average hormone concentration of the corpus luteum was 2.3 mcg. per gm. during Stage 1, increased significantly to 5 mcg. per gm. (P=0.01) during Stage 3 and decreased to 1.1 mcg. per gm. by Stage 7. Gawienowski (1956) has reported a range of 5.9 to 37.9 mcg. per gm. of luteal tissue in the pregnant sow (55 days). Loy *et al.* (1958) have investigated the hormone content of the corpora lutea of pregnant gilts (70 days) and found a correlation of 0.00 between the amount of luteal tissue and progesterone concentration. The level of progesterone in the

corpus luteum of the ewe (Edgar, 1953) is similar to that observed in this study of the pregnant cow.

The progesterone in residual ovarian tissue was 1.1 mcg. per gm. during Stage 1 and showed a significant increase to 2.7 mcg. per gm. during Stage 3 ($P=0.05$). A value of 0.8 mcg. per gm. was found in Stage 7. A hormone level of 16.0–23.9 mcg. per gm. was reported by Gawienowski (1956) for the same tissue from the pregnant sow (55 days).

The initial progesterone concentration in placental tissue was 0.08 mcg. per gm. and increased to 0.20 mcg. per gm. during Stage 4. There was a decrease to 0.03 mcg. per gm. (Stage 7). In the case of the mare, Short (1957) has reported a concentration of 0.073 mcg. per gm. of placenta from animals 120 days pregnant and 0.250 mcg. per gm. at 270 days.

The concentration of progesterone in allantoic fluid from the cow was found to be 0.06 mcg. per gm. during Stage 1 and increased to 0.08 mcg. per gm. during Stage 2. There was a decrease to Stage 6 (table 1). The hormone in bovine amniotic fluid varied during pregnancy from 0.01 to 0.04 mcg. per gm. Zander and von Münstermann (1954) have reported that progesterone ranges from 0.01 to 0.05 mcg. per ml. of human amniotic fluid. The hormone in the left adrenal gland varied from 0.4 to 2.1 mcg. per gm. (table 1). Beall and Reichstein (1938) have isolated small amounts of progesterone from the bovine adrenal.

As shown in table 2 the progesterone content of the corpus luteum and residual ovarian tissue was at a maximum during Stage 3 and then decreased during the remainder of pregnancy. These data represent the progesterone content of these organs at the time of slaughter and do not give a true evaluation of the rate of synthesis and release of hormone by these organs. The data in table 2 show a decrease in the amount of progesterone present in the corpus luteum of the cow during the latter part of pregnancy. However, Melampy and Gay (1958) observed no relationship between the weight of the corpus luteum and the stage of pregnancy in the cow. The progesterone of the placenta increased from 1 mcg. in Stage 1 to 403 mcg. in Stage 5 (table 2). This was due to an increase in hormone concentration (Stage 1-Stage 5, table 1) as well as the rapid development of the placenta during these stages. A significant decrease was observed in this organ between Stages 5 and 7 ($P=0.01$). The hormone content of allantoic fluid and the left adrenal gland reached a maximum during Stage 5 whereas this was observed during Stage 6 for amniotic fluid (table 2).

As shown in table 3 the average progesterone concentration in blood (mcg. per 100 ml.) increased from 0.9 mcg. (Stage 1) to 4.0 mcg. (Stage 6). A value of 3.1 mcg. was obtained during Stage 7. Short (1958) observed progesterone levels ranging from 0.74 to 0.98 mcg. per 100 ml. in bovine plasma from the 32nd to the 256th day of gestation. Following the 256th day of pregnancy the hormone level declined and the day prior to parturition the concentration was 0.0–0.4 mcg. per 100 ml. of plasma.

Edgar and Ronaldson (1958) found no progesterone in the peripheral blood of sheep; however, a hormone level ranging from 0 to 3.7 mcg. per ml. was observed in the venous drainage from the ovary during pregnancy in the ewe. These investigators were unable to detect the hormone in the blood shortly before parturition. Using the bio-assay of Hooker and Forbes (1947), Neher and Zarrow (1950) found that the progestin concentration rose from 1 to 2 mcg. per ml. at mating to a peak of 8–12 mcg. at parturition. They also reported higher hormone levels in ewes near the end of pregnancy. In the case of pregnant goats, Raeside and Turner (1955) noted the presence of progesterone in blood leaving an ovary that contained a corpus luteum and also in that from one containing a Graafian follicle. Furthermore, they were not able to detect the hormone in the venous

TABLE 3. CONCENTRATION OF PROGESTERONE IN WHOLE BLOOD FROM PREGNANT COWS

Stage	Duration of pregnancy in days	Number of samples	Mcg. progesterone per 100 ml. whole blood [a]
1	10–49	3	0.9 0.6
2	50–89	3	1.4 0.6
3	90–129	11	0.4 0.1
4	130–169	6	3.1 1.1
5	170–209	5	3.4 1.6
6	210–249	3	4.0 1.3
7	250–280	7	3.1 1.4

[a] Means and standard errors, ±.

drainage of the uterine horns, gravid and non-gravid, and in the peripheral blood of several large domesticated animals.

The data presented in tables 1, 2 and 3 are of considerable interest because several investigators have demonstrated the importance of a functional corpus luteum as well as the hormone progesterone during pregnancy in the cow. Hess (1921) and Schmaltz (1912) have stated that the removal of the corpus luteum is followed by abortion in the pregnant cow. Hammond (1927) observed that the corpus luteum maintains its size and shows no signs of involution during gestation. Furthermore, since a cow does not come into estrus for about 30 days after parturition, Hammond (1927) concluded that the atrophy of the corpus luteum is delayed until that time. The progesterone requirements for embryonic survival in the cow have been studied by Raeside and Turner (1950), and Uren and Raeside (1951). McDonald *et al.* (1952) reported that cows aborted in the absence of progesterone replacement therapy if the corpora lutea were removed between the 92nd and 163rd day of gestation. Pregnancy was maintained by injection of 100 mg. of progesterone daily in cows in which corpora lutea were removed at approximately the

60th day of pregnancy. In animals in which the corpora lutea had been removed, abortion did not occur when the hormone injections were discontinued as early as the 162nd day of gestation. McDonald *et al.* (1953) also found that cows maintained early pregnancies after corpus luteum ablation when aqueous suspensions of 500 mg. of progesterone were given at 10-day intervals.

Summary

A chemical study has been made of the progesterone concentration of the corpus luteum, residual ovarian tissue, placenta, allantoic and amniotic fluids and blood of the cow during pregnancy. The corpus luteum contained the greatest concentration of progesterone and residual ovarian tissue the next greatest. During the first 49 days of gestation the average progesterone concentration (mcg. per gm.), on a fresh-weight basis, was 2.3 for luteal tissue, 1.1 for residual ovarian tissue, 0.08 for placenta, 0.06 for allantoic fluid and 0.02 for amniotic fluid. During the interval between 90–129 days the concentration was 5.0 for luteal tissue, 2.7 for residual ovarian tissue, 0.16 for placenta, 0.07 for allantoic fluid, 0.04 for amniotic fluid and 1.7 for adrenal gland. During the final 30 days of pregnancy the concentration was 1.1 for luteal tissue, 0.8 for residual ovarian tissue, 0.03 for placenta, 0.04 for allantoic fluid, 0.02 for amniotic fluid and 0.7 for adrenal gland.

The progesterone contents of the corpus luteum and of the residual ovarian tissue were greatest during the period between 90–129 days of gestation. The placental content was at a maximum during the interval between 170–209 days. The largest amount of hormone was present in the allantoic fluid between 170–209 days and in the amniotic fluid between 210–249 days. The progesterone content of the adrenal gland reached a maximum during the period between 170–209 days of pregnancy.

The progesterone concentration in whole blood ranged from 0.9 mcg. per 100 ml. during early pregnancy to 4.0 mcg. at about the eighth month of gestation.

The presence of progesterone in purified extracts of reproductive tissues and fluids was confirmed by a positive Zimmerman reaction and infra-red absorption spectra indicating a carbon –20 carbonyl group and a $\triangle$–4, 3 keto group characteristic of progesterone.

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THE RELATIONSHIP OF CERTAIN BLOOD CONSTITUENTS TO RATE AND ECONOMY OF GAIN IN BEEF CATTLE

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RATE of gain and efficiency of feed utilization are important selection criteria in beef cattle breeding. Estimates of these performance characteristics are presently obtained from feeding tests extending over a period of several months. Accurate methods of determining inherent performance levels for these traits at an early age and in a short interval of time would result in a considerable saving in time and costs. Because blood fulfills a number of essential functions in the biological processes of growth and development, the variations in blood constituents were considered as possible criteria of rate and economy of gain. This study was undertaken to evaluate certain blood constituents as possible selection criteria for increased rate of gain and more efficient feed use.

Materials and Methods

Forty bulls (6 Angus, 22 Hereford, 12 Shorthorn) were included in the first year of the study, 1952. The bulls were weaned in the fall at approximately 6 months of age; after a 28-day adjustment period they were self-fed individually for 168 days on a ration containing about 68% T.D.N. A blood sample was collected from the jugular vein of each bull approximately 70 days after the start of the feeding test. This sample will be referred to as "intermediate" sample in the tables and discussion to follow. A second sample of blood was obtained from each bull within 6 days of the end of the 168-day test. Determinations of levels of glucose and sulfhydryl, and erythrocyte counts were made as soon as possible after collection of the blood. The methods used in making these evaluations and determinations as well as the ones mentioned later, have been described by Schultze (1955).

The 1953 data include 43 bulls (3 Angus, 31 Hereford, 9 Shorthorn). Feeding and management were the same as for the 1952 bulls. The first blood samples were collected within 8 to 14 days after the start of the feeding test. A second blood sample was obtained from each bull approximately 55 days after the first bleeding; this sample is referred to as

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"intermediate sample" in the tables. Hemoglobin content, hematocrit value, and eosinophil counts were obtained in addition to the three components studied the previous season.

Correlations were obtained from the within sire portions of the variances and covariances of the blood components, age, weight, and rate and efficiency of gain. The degrees of freedom within sires were 31 in 1952 and 33 in 1953.

Contemporary gain was measured during the 28-day period within which a respective blood sample was collected. Total gain and efficiency of gain were measured for the entire 168-day test. Efficiency was calculated as pounds of T.D.N. required per 100 lb. of gain; thus a negative

TABLE 1. MEANS AND STANDARD DEVIATIONS OF BLOOD COMPONENTS AND MEAN AGE AND WEIGHT

	Start of test 1953		Intermediate in test 1952		Intermediate in test 1953		End of test 1952	
Component	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Erythrocyte count (1000's per mm.[a])	8339	1090	8031	1087	9011	965	8369	1005
Glucose, mg./100 ml.	61	11.4	77	4.9	58	6.7	72	6.4
Sulfhydrl, mg./100 ml.	138	19.9	149	9.8	153	23.6	161	12.9
Esoinophil count, no./mm.[a]	228	201.0			115	67.2		
Hemoglobin, gm./100 ml.	11.1	1.0			12.2	1.3		
Hematocrit value, vol. %	35.4	3.9			39.2	4.2		
Age, days	219		280		273		381	
Weight[a] lb.	427		553		530		753	

[a] Taken at 28-day periodic interval closest to time of bleeding.

correlation between efficiency and another factor is interpreted as an association with more efficient feed use. Age was determined as of the start of the feeding test. Weight, as used in the analysis, was the 28-day periodic weight taken nearest the day a blood sample was collected.

The correlations between each blood component and total gain from all sampling periods were combined to obtain an estimate of the population correlation using Fisher's Z transformation. The same procedure was followed with the sample correlations between each blood component and efficiency of feed use. A table of chi-square distribution was used to test the hypothesis that the correlations were drawn from a common population of correlations. For several of the blood components the probability that the sample correlations were from a common population of correlations was less than 0.50; nevertheless, the average values are are presented as measures of the relative accuracy of the various individual blood components as criteria of rate and efficiency of gain.

Results and Discussion

Glucose. The means and standard deviations of glucose level measured at two intervals in each of two years are given in table 1. Glucose levels

in 1952 were higher than those in 1953, even when measured at the same stage in the feeding test. MacDonald *et al.* (1956) found blood sugar levels similar to the 1953 data, while Colby *et al.* (1950) reported values intermediate to the 1952 and 1953 data. In dairy cattle of comparable ages Schultze (1955) found glucose levels similar to the 1953 data, but considerably below those observed in 1952.

Correlation coefficients showing the relationship between glucose level and other characteristics are given in table 2. In 1953 a high blood glucose level at the start of the test was indicative of an ability to gain rapidly during the 168-day feeding test; 19% of the variation in total gain was accounted for by variations in glucose level. Glucose level measured early in the test was also positively associated with contemporary gain, i.e., gain during the 28-day period within which a blood sample was taken; 44% of the variation in first period gain was associated with variations in blood glucose level. These results were not consistent. At later stages in the feeding tests in both years the correlations between blood glucose and contemporary gain were much lower. The average of the correlations between glucose levels and total gain for all four sampling intervals was 0.17, indicating that blood glucose had very little overall association with this performance trait. In dairy cattle Schultze (1955) also found a low correlation (—.05) between glucose level and gain from 100 days to 360 days of age.

These data indicate that blood glucose level is not an accurate measure of inherent gaining ability. Very little is known of the heritability of blood glucose level in beef cattle, but the heritability in dairy cattle has been found to be low. Stormont (1954) reported a heritability of 0.17 and Plum and Schultze (1958) found a heritability of 0. Because blood glucose level appears to be affected largely by environment it would not be expected to be consistently associated with postweaning gain which has been reported to be highly heritable by Koch and Clark (1955), Shelby *et al.* (1955), Warwick and Cartwright (1955), and others. Their estimates of heritability of gain range from about 40 to 65%, although some estimates have been lower (Kincaid *et al.*, 1952). The large environmental influence on blood glucose level was also evident from the low correlation between blood glucose level at the first bleeding and the level at the second bleeding; the correlation was 0.16 in 1952 and 0.38 in 1953. Gain during the first 28-day period of the feeding test was as effective as initial blood glucose level as an indicator of total gain; the correlation was 0.45 compared to 0.44 for blood glucose.

A high glucose level was associated with more efficient feed utilization at both intervals in 1953, the correlation being —.41 in both instances. A negative correlation indicates efficiency because efficiency was expressed as pounds of T.D.N. required per lb. of gain. The corresponding correlations in 1952 were small, positive, and not statistically significant. The average of the four correlations was low (—.14).

Blood glucose level was not affected consistently by age of the bulls as seen in table 2. At the start of the test in 1953 glucose level was positively correlated with age, however, the means (table 1) indicate little change in blood sugar as the bulls advanced in age during the feeding tests. Schultze

TABLE 2. CORRELATIONS BETWEEN CERTAIN BLOOD CONSTITUENTS AND RATE AND EFFICIENCY OF GAIN, AGE, AND WEIGHT

	Sampling time during tests				
		Intermediate			
Correlations between	Start 1953	1952	1953	End 1952	Average
168-day gain and:					
Glucose	0.44**	0.06	0.17	—.04	0.17[1]
Sulfhydryl	.19	.01	.09	—.12	.05
Erythrocytes	.01	.07	—.12	.13	.02
Hematocrit	.04		—.14		—.05[1]
Hemoglobin	.13		.14		.13
Eosinophils	—.29		.03		—.14[1]
Contemporary 28-day gain and:					
Glucose	0.66**	0.13	—.34*	—.03	
Sulfhydryl	—.02	.10	.08	—.07	
Erythrocytes	—.15	.17	.27	.28	
Hematocrit	.14		—.05		
Hemoglobin	.13		—.13		
Eosinophils	—.45**		.35*		
Lb. TDN/100 lb. gain and:					
Glucose	—.41**	0.18	—.41*	0.17	—.14[1]
Sulfhydryl	—.12	.01	.09	—.10	—.03[1]
Erythrocytes	.07	—.22	.30	—.07	.03[1]
Hematocrit	—.33*		.15		—.10[1]
Hemoglobin	—.30		—.07		—.19[1]
Eosinophils	.31		.34*		.33
Age (days) and:					
Glucose	0.33*	0.17	—.26		
Sulfhydryl	—.10	.16	—.02		
Erythrocytes	—.24	.23	—.08		
Hematocrit	—.03		—.09		
Hemoglobin	—.09		—.02		
Eosinophils	—.11		—.13		
Initial weight and:					
Glucose	—.20				
Sulfhydryl	.40*				
Erythrocytes	.26				
Hematocrit	.18				
Hemoglobin	.12				
Eosinophils	.28				

* $P<0.05$.
** $P<0.01$.
[1] $P<0.50$ that the correlations are drawn from a common population of correlations.

(1955) found that glucose levels decreased with increasing age in dairy cattle, but in dairy cattle of comparable ages to the beef bulls studied here average glucose levels were quite constant. The association between initial weight and initial blood sugar level (table 2) was low.

Sulfhydryl. Kidwell *et al.* (1955) review several earlier studies indicating that glutathione is associated with rate of cell proliferation, protein synthesis and adult size. Because of this possible association with growth, sulfhydryl, which is generally considered to be an indirect measure of glutathione, was included in the present study.

The correlation coefficients in table 2 show that there was little relationship between blood level of sulfhydryl and contemporary gain, total gain on test, or efficiency of gain at the sampling intervals considered during both years of the study. These data are in agreement with the conclusions of Kunkel *et al.* (1954) and with the findings of Kidwell *et al.* (1955), except that the latter workers found a small, but significant, correlation between glutathione level and subsequent economy of gain. Schultze (1955) found that in dairy calves sulfhydryl was significantly related to gain ($r=-.51$).

Age had no appreciable effect on sulfhydryl level. Kunkel *et al.* (1954) made a similar observation concerning glutathione level. Their estimates of repeatabilities of glutathione (0.74 to 0.97) measured at different ages were higher than the repeatabilities of sulfhydryl found in the present study. The correlation between intermediate sulfhydryl level and final sulfhydryl level in 1952 was 0.50; in 1953 the correlation between initial level and intermediate level was 0.42. Schultze (1955) found that age of dairy calves had a significant affect on sulfhydryl level; however, most of the drop in sulfhydryl level came before the calves were 7 months of age. At ages comparable to those of the bulls studied here average sulfhydryl levels changed little with age.

A statistically significant correlation of 0.40 between sulfhydryl level and initial weight was obtained from the 1953 data. Kidwell *et al.* (1954) obtained a lower correlation (0.21) between glutathione and initial weight, but a correlation of similar magnitude (0.42) between glutathione and initial heart girth. Heart girth was considered more suitable than weight as measure of total size for studies of growth.

The 1953 data show that a large part of the blood sulfhydryl was found in the erythrocytes; 23 to 35% of the variation in sulfhydryl level was accounted for by the variation in total erythrocyte count, 31 to 36% by variation in hemoglobin level, and 48 to 61% by hematocrit value. Similar observations were made by Kidwell *et al.* (1954), who found that 23 to 30% of the variation in gluthathione was accounted for by total cell count or hemoglobin, by Kunkel *et al.* (1954) who found a correlation of 0.50 between hemoglobin and gluthathione in Herefords, and Schultze (1955) who reported a correlation of 0.47 between sulfhydryl and hematocrit. The correlations between erythrocyte count and sulfhydryl were much lower at both intervals in 1952 (0.19 and 0.22).

Erythrocyte Count, Hematocrit Value and Hemoglobin. These data are given in table 2. None of these three measures of the red cell fraction of blood was of value for predicting total gain on test or as indicators of past performance; the correlations of these items with total gain were in the range —.14 to 0.14. In dairy cattle Schultze (1955) found correlations of —.32 to —.38 between these three blood components and gain from 100–360 days of age. In the present study low relationships were found between erythrocyte count, hematocrit, and hemoglobin and contemporary gain, i.e., gain during the 28-day period within which the blood sample was obtained. The correlation coefficients range from —.15 to 0.28; none was statistically significant.

At some of the intervals in which the determinations were made, red cell count, hematocrit and hemoglobin were associated with more efficient gain, but these results were not consistent. The average effect of any one of these items on gain was slight; the average correlations varied from 0.03 to —0.19.

The number and volume of red cells, or the amount of hemoglobin in the blood of beef bulls seem to be influenced to a considerable extent by environmental factors, rather than being a constant, individual characteristics of a particular animal. Only 6 to 28% of the variation in these items at a second sampling was accounted for by the variations in the same item sampled at an earlier part of the feed test. These results are not in agreement with the results with dairy cattle; Schultze (1955) observed a significant difference between individuals for all of these blood characters. Plum and Schultze (1958) also found heritabilities of 0.5 and 0.7 for hemoglobin and R.B.C., respectively. Part of the lack of agreement between results with beef cattle and those with dairy cattle may be in inherent differences in the two types of cattle; some may also be due to differences in management and time of sampling.

Circulating Eosinophils. Schultze (1957) suggested that circulating eosinophil level might be related to an animal's ability to adjust to adverse conditions because of the inverse relationship between eosinophils level and the amount of circulating adrenocortical hormones; an animal's ability to adjust to adverse situations with a minimum expenditure of energy should enhance its ability to expend a maximum of energy in desired agricultural production.

Correlation data for counts of circulating eosinophils are shown in table 2. Low eosinophil counts at the start of the test were slightly indictive of more rapid gain (r=—.29) and greater efficiency (r=—0.31) during the 168-day test that followed. The correlation with total gain dropped to near zero when counts were made intermediate in the test; but the correlation with efficiency (r=0.34) remained about the same. The average correlation with total gain (—.14) was very similar to the correlation of —.13 reported by Schultze (1957) for dairy calves. Eosinophile count was the most closely associated with contemporary gain

TABLE 3. STANDARD PARTIAL REGRESSION COEFFICIENTS OF TOTAL GAIN ON EACH OF SEVERAL FACTORS AND MULTIPLE CORRELATION COEFFICIENTS BETWEEN TOTAL GAIN AND VARIOUS COMBINATIONS OF THESE FACTORS

Combination no.	Standard partial regression of total gain on—									Multiple correlation coefficient
	Erthrocyte count	Blood sugar	Sulfhydryl	Eosinophil count	Hemoglobin	Hematocrit	Age	Wt.	28-day gain	
1	—.01	0.28	0.34	—.23	0.17	—.39	0.14	0.21		0.57
2		.42	.38			—.28				.49
3		.42	.16							.46
4		.24							0.29	.49

of any of the blood constituents. However, the correlations, even though statistically significant, are too inconsistent (—.45 to 0.35) to be readily explained. Eosinophil count also showed the highest average correlation with efficiency of gain of any of the blood constituents; even so, variations in circulating eosinophil on the average accounted for only 11% of the variation in efficiency.

Multiple Correlation. Certain standard partial regression coefficients and multiple correlation coefficients were calculated from the 1953 data; these are given in table 3. The blood components in the blood sample collected near the beginning of the test are the only ones included. A multiple correlation coefficient of 0.57 was obtained between total gain and a group of six blood constituents plus age and initial weight (combination No. 1). This multiple correlation is not much larger than the simple correlations discussed earlier between total gain and blood sugar (0.44), and between total gain and first period gain (0.45).

The standard partial regressions in combination No. 1, indicate that hematocrit, sulfhydryl, and blood glucose, in that order, made the largest independent contribution to variation in total gain. These three blood components were included alone in combination No. 2. The resulting multiple correlation was 0.49; these three items were only slightly more effective for predicting total gain than either blood glucose or first period gain. The third combination of factors in table 3, included blood glucose and sulfhydryl; this combination was only slightly more indicative of future gain on test (r=.46) than was blood sugar alone. Blood glucose and first period gain, the two best single indicators of total gain in the first sample in 1953, were considered as the fourth combination. Their combined predictive value (r=0.49) for gain was only slightly better than that of each item alone.

The previous discussions show that none of the blood constituents studied was closely associated with gain or efficiency of gain; or that if a close relationship with growth exists, interrelationships between these and other biological factors are too complex and interdependent for effects to be adequately evaluated by the type of study reported here.

Glucose level was the most accurate of the blood constituents for predicting gain and efficiency at one of the sampling times. However, glucose level was not consistently accurate at other intervals. This fact, plus additional evidence presented here and by other workers indicate that environmental factors have a great influence on blood glucose level.

The gain made by the bulls in the first 28 days of the tests was almost as useful in predicting gaining ability in a 168-day test as was a combination of the six blood constituents, age and initial weight. Gain during the first 28-day period was also a better indicator of efficiency of gain during the entire test period than any blood constituent; the correlation between first period gain and efficiency (TDN/100 lb. gain) was —.65.

Summary

Glucose, sulfhydryl and hemoglobin levels, hematocrit values, and circulating eosinophil and erythrocyte counts were determined for beef bulls at two intervals in each of two years during 168-day feeding tests subsequent to weaning. Averages of the correlations between each blood constituent and each of two performance traits were obtained. The average correlations between total gain and individual blood constituents varied from —0.14 (with circulating eosinophils) to 0.17 (with blood sugar level). The average correlations between efficiency of feed use and individual blood constituents were in the range —0.19 (with hemoglobin) to 0.33 (with circulating eosinophil count). None of the individual blood constituents was an accurate indicator of these two performance traits.

Multiple correlation analysis showed that 33% of the variation in total gain was accounted for by variations in the six blood constituents measured at the start of the test, plus variations in age and initial weight. Various combinations of the blood constituents were only slightly more accurate as indicators of total gain than first 28-day period gain. First period gain was associated with 20% of the variation in total gain and 42% of the variation in efficiency of feed use.

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ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS IN BEEF CATTLE

II. Heritability Estimates from Parent-Offspring and Half-Sib Resemblances [1]

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IN a recent paper (Kincaid and Carter, 1958) the authors presented results from an experiment designed to measure progress to be achieved from sire selection and to estimate directly heritability of postweaning growth rate of beef cattle. Sires, selected in equal numbers for rapid and slow rates of gain on a 168-day feeding test following weaning, were mated to random samples of a grade cow herd for progeny test. Progress from selection and heritability of rate of gain were measured by differences between the gains of the progeny of the two groups of sires.

Heritability of only one trait, postweaning growth rate, could be estimated from the results from selection among the sires. It is of importance, however, to obtain estimates of heritability of other economically important characters. It is also of interest to compare estimates of heritability of the same trait obtained indirectly from resemblances among relatives as compared with nonrelatives. Estimates of heritability of a number of traits, derived from parent offspring and paternal half-sib comparisons are presented herein.

Analysis of the Data and Results. The traits studied in the steers were: Weight at 6 months (182 days) of age, feeder grade at weaning, daily gain in feedlot, T.D.N. requirements per cwt. of gain, slaughter grade and carcass grade. In the heifers the traits were weight at 6 months, feeder grade at weaning, daily gain on pasture and yearling feeder grade. Phenotypic means in each of these traits for the selected high and low gaining sires and their progenies are shown in tables 1 and 2.

All calves were weighed at 14 or 28 day intervals during the suckling period. No supplemental feed was fed, either to the cows or calves during this period. Weights were adjusted to a standard age of 6 months (182 days) by linear interpolation between adjacent weights taken just before and after the 182nd day of age of the calf. The steers were placed on full feed for a 200-day feeding period immediately after weaning with the unadjusted weaning weight taken as the initial weight. T.D.N. requirements per cwt. gain were adjusted for differences in average weight by covariance analysis. The heifers were wintered largely on roughage and grazed on bluegrass pasture their yearling summer. Daily gains on pasture

[1] Contributing to Southern Regional Project, S–10.

TABLE 1. PHENOTYPIC MEANS OF SELECTED HIGH AND LOW GAINING BULLS

Items	High gaining	Low gaining	Standard deviation[a]
Initial weight	572	582	91.1
Daily gain	2.24	1.65	.28
T.D.N. per cwt. gain	558	682	83
Initial type score[b]	11.6	11.2	1.29
Final type score[b]	11.8	10.8	1.69

[a] Standard deviations within year, breed, and age groups among all bulls on test with 121 d.f.
[b] Grade or type score: Prime or Fancy 15–17; Choice 12–14; Good 9–11; Medium or Commercial 6–8; Utility or Common 3–5.

during this period (April 20-October 10) constituted the gain evaluation test of the heifers.

Feeder and slaughter grades were the averages of the grades of a committee of three to five working independently. Carcasses were graded by the official U.S.D.A. meat graders at the packing plant at which the steers were slaughtered.

Paternal Half-Sib Correlations. Records on the progeny of 38 sires accumulated over a 6-year period were available for half-sib analysis. These include weanling observations on 212 steers and 212 heifer calves, feedlot and slaughter data on 192 steers, and yearling records on 202 heifers. Not all the steers were fed individually—only 107 steers in four of the years. The progeny of 26 sires were available for analysis of T.D.N. requirements per cwt. gain.

The cows were rerandomized to the various sire groups each year without regard to grouping in previous years. After breeding season the cows and calves from the various breeding lots were grouped and run together for the remainder of the year. Thus, environmental correlations among

TABLE 2. PHENOTYPIC MEANS OF STEER AND HEIFER PROGENY OF SELECTED HIGH AND LOW GAINING BULLS

	Steer progeny				Heifer progeny			
	High		Low		High		Low	
Items	No.	Av.	No.	Av.	No.	Av.	No.	Av.
Six-month weight	106	395	96	385	103	375	99	356
Feeder grade[b]	106	11.6	96	11.6	103	12.2	99	12.2
Daily gain	91	1.81	86	1.71	96	1.57	96	1.51
Slaughter grade[b]	91	12.4	86	12.3				
Carcass grade[b]	87	11.7	83	11.5				
T.D.N. per cwt. gain	57	645	50	688				
Yearling feeder grade[b]					103	11.7	103	11.5

[b] See grade or type score under table 1.

TABLE 3. STEERS, HERITABILITY ESTIMATES BY PATERNAL HALF-SIB METHOD

Items	Among sires		Within sires		k[a]	σ_s^{2b}	Heritability estimate
	DF	MS	DF	MS			
Six-month weight	23	1627	162	1446	5.72	31.6	0.08
Feeder grade	23	3.70	161	2.27	5.49	.26	.41
Daily gain (feedlot)	23	.0597	138	.0378	5.49	.004	.38
T.D.N. per cwt. gain	18	9930	78	3610	5.35	1181	.99
Slaughter grade	23	2.15	138	1.27	5.35	.16	.45
Carcass grade	23	1.58	130	1.26	4.78	.067	.20

[a] k=no. offspring per sire.
[b] σ_s^2=sire component of variance.

progeny of one sire relative to other sire progenies should be negligible. The effects of differences due to year, age of dam, and breed of sire were removed by analyzing the data within year, breed of sire and age of dam groups. The resulting within group sums of squares and degrees of freedom were pooled to estimate the variance components. Since the heifers were treated differently from the steers following weaning, the two sexes were analyzed separately and estimates of heritability obtained for each group.

The accuracy of heritability estimates from half-sib data depends on the number of degrees of freedom for sires (and to a lesser degree on the number of offspring per sire), on the absence of environmental correlations among the half-sibs relative to the non-sibs and on the absence of selection, among the sires. The method is theoretically valid only if the sires can be considered a random sample of some unselected population. In the present study, since the sires were selected for both extremes in daily gain, the paternal half-sib heritability estimate would be expected to be biased upward in so far as the selection were effective in separating the progeny means.

Estimates of heritability for all traits studied were obtained from paternal half-sib correlations even though it is realized that sire selection may have introduced bias into some of them. These are shown separately for the steers and heifers in tables 3 and 4.

Parent Offspring Regression. Heritability estimates from regression of offspring on parent are usually considered more reliable than those based

TABLE 4. HEIFERS, HERITABILITY ESTIMATES BY PATERNAL HALF-SIB METHOD

Items	Among sires		Within sires		k[a]	σ_s^{2b}	Heritability estimate
	DF	MS	DF	MS			
Six-month weight	23	3161	166	1489	5.37	311.5	0.69
Feeder grade	23	4.01	166	2.25	5.37	0.328	.51
Daily gain (pasture)	31	0.0698	153	0.0389	5.08	0.0068	.54
Yearling grade	31	2.65	152	2.17	5.08	0.094	.17

[a] k=no. offspring per sire.
[b] σ_s^2=sire component of variance.

TABLE 5. STEERS. HERITABILITY ESTIMATED BY REGRESSION OF PROGENY AVERAGE ON SIRE

Character		D.F.				Herit-
Progeny	Sire	sires*	ΣX_1X_2**	ΣX_1^2**	b±s.e.	ability
Feeder grade	Initial grade	25	4.71	60.40	0.08±0.09	0.16
Daily gain	Daily gain	28	.43	4.07	.11± .09	.21
T.D.N./cwt. gain	T.D.N./cwt. gain	28	47832	436147	.11± .09	.22
Slaughter	Final grade	28	4.06	119.96	.03± .06	.07

* Degrees of freedom for sires within years and stations.
** X_1 refers to trait X in the parent, X_2 to trait X in the offspring.

on half-sib data. In both methods the observed covariance between the phenotypes of the relatives is divided by the known correlation between their genotypes, i.e., the genetic relationship. Under random mating this relationship is one-half between parent and offspring and one-fourth among half-sibs. Thus the regression of offspring on parent is doubled to obtain the heritability estimate but the correlation among half-sibs is multiplied by 4. Sampling and non-random errors are thus inflated by only a factor of 2 in the case of parent offspring comparison, but by 4 with half-sib estimates.

The parent-offspring method excludes the effects of environment more effectively than those based on full or half-sibs. The parent and offspring are not contemporary and are thus not subjected to the same environment as may be the case with sibs. The regression method is not biased by selection among the parents as is the case with the half-sib correlation method. In the present study the cows were an unselected group but the sires were highly selected for extremes in daily gain as was mentioned above.

Heritability may be estimated using the regression of the offspring on either parent. In large animal data, such as these, the dams far outnumber the sires. Since the accuracy of the estimate depends largely on the number of parents rather than the number of offspring per parent, the estimate is usually obtained from the regression of offspring on dam. In the present study observations were available on the dams only for feeder grade at weaning, daily gain on pasture and yearling feeder grade. In feeder grade, 152 dam-offspring pairs were available among

TABLE 6. STEERS, HERITABILITY ESTIMATED BY INTRASIRE REGRESSION OF OFFSPRING ON DAM

Offspring	Dam	Dam/ Offspring pairs	ΣX_1X_2**	ΣX_1^2**	b±s.e.	Herit- ability
Feeder grade	Feeder grade	152	29.48	786.54	0.04±0.05	0.07
Daily gain (feedlot)	Daily gain (pasture)	97	1.21	6.23	.20± .06	.40

** X_1 refers to trait X in the parent, X_2 to trait X in the offspring.

TABLE 7. HEIFERS, HERITABILITY ESTIMATED BY REGRESSION OF PROGENY AVERAGE ON SIRE

Character						
Progeny	Sire	D.F. sires *	ΣX_1X_2 **	ΣX_1^2 **	b±s.e.	Herit-ability
Feeder grade	Initial grade	25	18.91	60.40	0.31±0.10	0.63
Daily gain (pasture)	Daily gain (feedlot)	28	.40	4.08	.10± .02	.20
Yearling grade	Final grade	28	.72	61.95	.001± .03	.002

* Degrees of freedom for sires within years and stations.
** X_1 refers to trait X in the parent, X_2 to trait X in the offspring.

the steers and 158 pairs among the heifers; for daily gain, 97 pairs with steers and 133 with heifers and 133 daughter-dam pairs for yearling feeder grade. Heritability estimates for these traits from intra-sire regression of offspring on dam are shown in tables 6 and 7.

Observations were available for several traits in the sires including initial grade, gains on feed test, T.D.N. requirements per cwt. gain and final grade. Regression of progeny average on sires records were calculated, within year and station, to obtain heritability estimates. These are shown in tables 5 and 8.

Discussion

Perhaps the parameter of greatest interest and importance in improvement of beef cattle at present is heritability of postweaning growth rate or gains. In the previous paper (Kincaid and Carter, 1958) the authors estimated heritability of postweaning gains, from results from sire selection, to be about one-third (0.32 to 0.38 in the steers, 0.33 to 0.35 in the heifers). The half-sib estimate found in the present study is in close agreement with these estimates for the steers, 0.38, but is higher for the heifers, 0.54. The estimates obtained from offspring-dam regression are quite close to the half-sib estimates, 0.40 and 0.58 for the steers and heifers, respectively. The estimates obtained from regression of progeny average on sires daily gain are lower, 0.21 and 0.20 for the steers and heifers, respectively.

TABLE 8. HEIFERS, HERITABILITY ESTIMATED BY INTRASIRE REGRESSION OF OFFSPRING ON DAM

Items	Dam/ Offspring pairs	ΣX_1X_2 **	ΣX_1^2 **	b±s.e.	Herit-ability
Feeder grade at weaning	158	−66.40	904.05	−.07±0.05	0.0
Daily gain (pasture)	133	2.10	7.40	.28± .09	.57
Yearling feeder grade	129	62.37	384.46	.16± .02	.32

** X_1 refers to trait X in the parent, X_2 to trait X in the offspring.

These estimates are generally lower than most of those reported by other investigators (Knapp and Nordskog, 1946a; Patterson *et al.*, 1949; Shelby *et al.*, 1955) for heritability of feedlot gains with steers. Most of these were derived from half-sib correlations and ranged from 0.5 to 1.0. The estimates of Warwick and Cartwright (1955), making use of the "gain ratio" rather than absolute values, are somewhat lower, 0.33 to 0.51, and are closer to those found by us. Estimates from this study for heritability of yearling summer gains of heifers on pasture were closer to those reported by other investigators including 0.19 by Cartwright (1955) and those by Koch and Clark (1955) ranging from 0.18 to 0.39.

Heritability of weight at 6 months (182 days) was estimated from the half-sib analysis at 0.08 for steers and 0.69 for heifers. The average difference in weight at 6 months between the progeny of the high and low gaining sires was 10 lb. for the steer calves and 19 lb. for the heifers (table 2). Such a difference between the sexes in heritability of weaning weight has not been previously reported to the knowledge of the authors. A possible explanation may be the slower growth rate, and presumably lower nutritive requirements for optimum growth, of heifers compared to steers. Perhaps the available feed, milk, grass, was more nearly adequate for maximum expression of heritable differences in growth rate among the heifers than the steers. If this were true then the performance of heifers may give a better measure of the breeding value of a sire than that of steers, particularly where nutrition or environment was less than optimum.

Other estimates of heritability of weaning weight include 0.12 (Knapp and Nordskog, 1946a) 0.28 (Knapp and Clark, 1950) and 0.23 (Shelby *et al.*, 1955) all for Hereford steer calves at the Miles City, Montana, Range Station. Koch and Clark (1955) obtained estimates of 0.24 and 0.11, from half-sib and parent-offspring analyses respectively, for Hereford heifer calves at the same station and Gregory *et al.* (1950) reported estimates of 0.25 and 0.52 for Hereford calves of both sexes at two stations in Nebraska. Rollins and Wagnon (1956a) reported heritability of 240-day weaning weight of grade Hereford calves under range conditions in California at 0.30, and concluded that supplemental feeding of the cows, but not the calves, during the dry season did not affect the heritability of weaning weight of the calf.

Heritability of feeder grade at weaning was estimated at 0.41 for the steer calves and 0.51 for the heifers from half-sib correlations. Estimates obtained from offspring-parent regression were generally lower; for the steers and heifers respectively, 0.16 and 0.63 from regression of progeny average on sires initial grade and 0.07 and zero from regression of offspring on dam. A possible explanation for the low estimate obtained from the offspring-dam comparison was the rather coarse classification of feeder grade of the dams. The dams were graded only to full grades (Good, Choice, etc.) while the grades of the offspring were averages of scores of a committee of three or more, each of whom graded the calves by one-

third of a grade (High Good, Low Choice, etc.). The half-sib estimates found here are in the upper range of those by other investigators including 0.48 and 0.75 of Knapp and Nordskog (1946b); 0.23 and 0.50 (Koger and Knox, 1957); 0.18 (Koch and Clark, 1955); and 0.36 (Rollins and Wagnon, 1956b).

The estimate of heritability of feed efficiency (T.D.N. requirements per cwt. of gain) of 0.99 is obviously unrealistic. No explanation of this extremely high value is evident, but it is based on only 18 degrees of freedom for sires. Knapp and Nordskog (1946a) report heritability of efficiency of gain at 0.48 and Shelby *et al.* (1955) at 0.22. Heritability of slaughter grade was estimated at 0.45. Similar estimates of 0.45 and 0.42 were reported by Knapp and Clark (1950) and Shelby *et al.* (1955). The estimate of 0.16 for heritability of carcass grade is the same as that of Shelby *et al.* (1955). Knapp and Clark (1950), however, reported a somewhat higher figure, 0.33.

Summary

Heritability of a number of important economic traits in beef cattle was estimated from records on 424 calves, the progeny of 38 sires, raised over a 6-year period at the Virginia Station. Estimates obtained from paternal half-sib correlations were, for the steers: Weight at 6 months, 0.08; feeder grade, 0.41; daily gain in feedlot, 0.38; feed efficiency, 0.99; slaughter grade, 0.45; and, carcass grade, 0.16. For the heifers these were: Weight at 6 months, 0.69; feeder grade, 0.51; daily gain on pasture, 0.54; and, yearling feeder grade, 0.17. Heritability estimates calculated from regression of progeny average on sires' records were, for the steers: Feeder grade, 0.16; daily gain in feedlot, 0.21; feed efficiency, 0.22; slaughter grade, 0.07; and, for the heifers they were: Feeder grade, 0.63; daily gain on pasture, 0.20. Estimates calculated from intra-sire regression of offspring on dam were, for the steers; Feeder grade, 0.07; daily gain, 0.40; and, for the heifers they were: Feeder grade, zero; daily gain on pasture, 0.57; and, yearling feeder grade, 0.32.

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ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS IN BEEF CATTLE

III. Genetic and Phenotypic Correlations among Economic Characters [1]

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A KNOWLEDGE of the genetic and phenotypic relationships among the important economic characters in domestic livestock is important to establish a basis of selection for the simultaneous improvement of two or more characters. A number of investigators have studied phenotypic or gross correlations among various characters in beef cattle. These have included correlations among several linear body measurements and such production traits as weight, rate and economy of gain and feeder, slaughter and carcass grades and carcass yields. Others have included correlations among gains made during different periods or seasons. Only a few estimates of genetic correlations among traits in beef cattle have been published. Most of these have been concerned with relationships among gains in different periods and grades or scores.

Experimental

In the present study genetic and phenotypic correlations among various traits were estimated from data on 195 steers and 190 heifers, calved over a 5-year period, the progeny of 36 sires at the Virginia Station. The sires were selected in equal numbers for rapid and slow rates of gain on a 168-day performance feeding test following weaning. The selected bulls, 22 Hereford, 12 Angus and 4 Shorthorns, were mated to random samples of grade Hereford cows for the progeny test. The steer progeny were full fed individually on gain evaluation test for 200 days following weaning and were slaughtered at the end of the test. The heifers were wintered largely on roughage and tested by gains made on pasture their yearling summer. Details of the performance and progeny tests may be found in a previous paper in this series (Kincaid and Carter, 1958).

The traits studied in the steers were weight at 6 months (182 days); feeder grade at weaning; daily gain on feed; T.D.N. requirements per cwt. gain (adjusted for mean liveweight); slaughter grade and carcass grade. In the heifers, the traits were weight at 6 months; feeder grade at weaning; daily gain on pasture and yearling feeder grade.

Genetic correlations were estimated from paternal half-sib analysis, using the sire components of variance and covariance as described by Hazel

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TABLE 1. GENETIC AND PHENOTYPIC CORRELATIONS AMONG TRAITS IN STEERS

Items		Feeder grade	Daily gain	T.D.N./ cwt. gain	Slaughter grade	Carcass grade
182-day weight	Genetic	0.49	0.66	0.43	0.36	0.84
	Pheno.	.32	.24	.26	.28	.23
Feeder grade	Genetic		.28	—.11	.66	.65
	Pheno.		—.07	.11	.36	.16
Daily gain	Genetic			—.32	.48	.85
	Pheno.			—.50	.38	.31
T.D.N./cwt. gain	Genetic				.18	.16
	Pheno.				.08	.16
Slaughter grade	Genetic					.83
	Pheno.					.60

et al. (1943). Phenotypic relationships were obtained as product moment correlations using pooled intra-year and station variances and covariances. Estimates were obtained separately for the steers and heifers.

The sires in this study were not a random sample of sires, but were highly selected for rate of gain. It is possible that this selection might bias the estimates for traits correlated with daily gain although the nature or magnitude of this bias (if present) is not clear to the authors.

Results and Discussion

The genetic and phenotypic correlations, calculated separately for the steers and heifers, are shown in tables 1 and 2.

There appeared to be little relationship between grades and subsequent gains. In the steers the phenotypic correlation between feeder grade at weaning and daily feedlot gain was slightly negative (—.07) but a small positive genetic correlation was found (0.28). These correlations in the heifers were essentially zero. Other investigators have reported similar low phenotypic correlations between feeder grade and subsequent gain

TABLE 2. GENETIC AND PHENOTYPIC CORRELATIONS AMONG TRAITS IN HEIFERS

Items		Feeder grade	Daily gain	Yearling grade
182-day weight	Genetic	0.31	0.51	0.50
	Pheno.	.37	.20	.28
Feeder grade	Genetic		—.0004	.63
	Pheno.		—.02	.56
Daily gain	Genetic			.58
	Pheno.			.0002

Hankins and Burk, 1938, (0.09); Knapp and Clark, 1951 (essentially zero); Durham and Knox, 1953 (—.006 to 0.11). Knapp and Clark (1951) reported a genetic correlation of 0.3 between weaning score and feedlot gains.

Moderate to high positive genetic correlations were found between gains in a particular period and grade at the end of the period. These include weight at 6 months and feeder grade, 0.49 and 0.31, for the heifers and steers, respectively; daily gain on feed with live grade at slaughter (0.48) and with carcass grade (0.84) in the steers; and, summer pasture gain with yearling feeder grade (0.59) in the heifers. The respective phenotypic correlations were similar but generally somewhat smaller. These are in accord with similar correlations reported by Koch and Clark (1955) and Hankins and Burk (1938).

Rather high positive correlations, both genetic and phenotypic, were found among grades in different periods. The respective genetic and phenotypic correlations in the steers were feeder grade at weaning with live grade at slaughter, 0.66 and 0.36; feeder grade with carcass grade, 0.65 and 0.16 and slaughter with carcass grade, 0.86 and 0.60. In the heifers, the respective correlations for weaning with yearling feeder grades were 0.63 and 0.56. Other investigators (Koch and Clark, 1955; Hankins and Burk, 1938) have reported similar high correlations; those reported by Durham and Knox (1953) were somewhat lower.

A high positive correlation between rate and efficiency of gain, if adjusted for differences in gross body weight, has been reported by a number of investigators (Winters and McMahon, 1933; Black and Knapp, 1936; Knapp *et al.*, 1941; Stanley and McCall, 1945; Kohli *et al.*, 1951; Grizzle and Kincaid, 1954). This usual relationship was found in the present study as indicated by the negative genetic correlation of —.32 between rate of gain in feedlot and TDN requirement per cwt. of gain among the steers. The phenotypic correlation, —.50, is within the range of those found by other investigators although somewhat below the average of the reported values. A high correlation between rate and efficiency of gain, particularly in a population of equal gross body size, is largely automatic and is a reflection of the more or less constant maintenance requirements of animals of the same liveweight. In effect it is a ratio correlated with its denominator.

Perhaps the most important genetic relationship found in this study is the fairly high positive correlation between gain from birth to weaning, indicated here by weight at 182 days, and postweaning gains. The genetic correlation between weight at 6 months and feedlot gain was 0.69 in the steers, and in the heifers, 0.51 between 6 months weight and yearling gain on pasture.

The respective phenotypic correlations were 0.27 and 0.20. These estimates are quite different from those reported by Koch and Clark (1955). They found negative genetic and phenotypic correlations, —.05

and —.36, respectively, between gain from birth to weaning and yearling summer pasture gains in heifers. They concluded that gains from birth to weaning and postweaning gains were genetically independent.

The estimates of Koch and Clark (1955) were based on several times as much data as the present study and for this reason might be considered more reliable; however, it is possible that the differences in the correlations found in their study and this one may be the result of the different environments under which the cattle were raised. They used data from the U. S. Range Station at Miles City, Montana. Environmental conditions there are typical of the semi-arid Northern Great Plains. Cattle are kept on the range the year round with supplemental feed only during severe storms or droughts. The cattle in the present study were under much more favorable environmental conditions without the typical interruptions of growth that occur under range conditions during drought or in winter.

If the positive genetic correlation between postweaning and preweaning growth rate apparent in this study is real it will be of marked advantage to breeders in improving the growth potential of their animals. Selections can be made much more easily and economically at weaning than later. Selection among progenies for heavy weaning weight should lead to improvement in subsequent growth rates. Progeny testing could be largely based on preweaning performance without the necessity of expensive postweaning performance tests.

This seems to be of sufficient importance to justify further investigation with independent data to verify or refute the findings of this study.

Summary

Genetic and phenotypic correlations among various traits in beef cattle were estimated using data from 195 steers and 190 heifers born over a 5-year period, the progeny of 36 bulls.

No negative genetic correlations that would handicap selection are evident among the traits studied. The genetic correlations were all positive except those between grades and subsequent growth rates which appear to be independent genetically, and a negative correlation between rate of gain and TDN requirements per unit gain. This of course could be expressed as a positive correlation between rate and efficiency of gain.

High positive correlations were found between gains in a particular period and grade at the end of the period. Slaughter and carcass grades have high correlation and the correlations between feeder grade at weaning and slaughter, carcass or yearling feeder grade are moderately high.

Perhaps the most important genetic correlation found was that between preweaning growth rate and subsequent rate of gain in the feedlot or on pasture. This was estimated to be 0.69 for the steers and 0.51 for the heifers. Improvement in both traits should result from selection for either.

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INFLUENCE OF PELLETING ON THE UTILIZATION OF ALFALFA

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IMPROVEMENT in harvesting, transporting, storing and feeding of hay not only influences the economics of hay handling but may influence the feeding value of hay. Pelleting is such a procedure. In general, particularly when the largest proportion of the ration was hay, increased rate of gain resulted when the ration was pelleted (Neale, 1953; Cate *et al.*, 1955; Esplin *et al.*, 1957; Ittner *et al.*, 1958). When larger gains were observed on pelleted feeds an increased feed consumption was generally found. Further studies showed that pelleted and chopped hay differ in their rate of passage through the digestive tract (Blaxter and Graham, 1956; Blaxter *et al.*, 1956). Pelleted hay had a faster rate of passage through the digestive tract and had a lower digestible and metabolizable energy content but was equal to chopped hay in net energy because of a lower heat increment.

The purpose of these experiments was to investigate the reasons for an increased feed intake when alfalfa hay is finely ground and compressed into a pellet.

Experimental

A uniform field of alfalfa (16% of the stems had one or more buds and 84% of the stems were pre-bud) was field chopped and dehydrated. A representative amount of the chopped hay was sacked while the remainder was ground through a 3/64 inch screen in a hammer mill and made into 3/8 inch pellets.

Two feeding trials were conducted and both involved the pair-feeding technique. The first experiment contained two groups—chopped and pelleted hay pair-fed at a maintenance feeding level; the second experiment contained three groups—chopped hay fed *ad libitum*, pelleted hay pair-fed to the chopped hay fed group and pelleted hay fed *ad libitum* (table 3). Therefore, pelleted hay was compared to chopped hay on an equalized feed intake basis at two levels of feeding. The wether sheep were carefully selected and assigned to groups on the basis of weight and degree of finish. Representative animals equivalent in size and finish were killed for initial body composition. All body weights were taken after

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24 hours without feed and 14 hours without water. Animals were fed individually. The feed was sampled as fed, and dry matter determined weekly on the composited sample.

A digestion trial was conducted with the sheep which were fed at the maintenance level. Seven-day preliminary and seven-day collection periods were used during which feed intake was held at a constant level. Feces were collected daily, dried in a forced-air oven at 70° C. for 24 hr. and the entire seven-day collection mixed, ground and quartered into the laboratory sample. Energy determinations were made in an Emerson fuel calorimeter. A known amount of urine was concentrated on a weighed quantity of cellulose powder to determine its energy content. Methane production was computed from dry-matter intake by a formula presented by Swift and French (1945).

Prior to the digestion trial the sheep fed at the maintenance level were used as sources of rumen contents for a study of fatty acids, carbon dioxide and methane production. For one week an equal quantity of ration was fed at 12-hour intervals to each pair. Rumen collections were made with a stomach tube according to a predetermined schedule at 0, 1½, 4 and 8 hr. after feeding. Collections were made at the same time from both members of a pair fed chopped and pelleted hay. Twelve days were needed for this series of tests in order for the 12 sheep to supply rumen contents at each time after feeding but at least 24 hr. elapsed between sampling of any one individual pair.

It required approximately 10 min. to collect the rumen contents, another 15 min. before incubation in the Warburg and readings were started within one hour from collection and continued for one hour. The Warburg manometer technique, incubating whole rumen contents, as described by Hungate *et al.* (1955) was used. The technique was slightly modified: 20 gm. of rumen sample was used with 40 ml. of 0.5% sodium bicarbonate buffer in each flask and the time of gassing was reduced to 3 min. when the flasks were not shaken and 4 min. when the flasks were shaken. The samples were run in quadruplicate, two control and two experimental flasks. A lack of agreement between duplicates necessitated repeating certain runs.

Seven days before the end of the feeding trial all pair-fed sheep in both experiments were fed at 12-hour intervals, then salughtered and the contents of their digestive tracts studied. Three pairs were killed at 0, 1½, 4 and 8 hr. after feeding. As soon as each sheep was dead the reticulorumen, omasum, abomasum and small intestine were removed. Each was weighed, the contents removed, and the weight of the contents determined by difference. The content of each organ was sampled and dried at 70° C. in a forced air oven. The samples were then ground for nitrogen, holocellulose, lignin and ash analyses.

The carcasses of the sheep after chilling were used for specific gravity determinations. Fat and energy content of the carcass was then calculated

as described by Garrett *et al.* (1958). Net energy of the feed was then calculated since two levels of feeding were used. The difference in the energy gain of these groups fed at two levels of feeding was divided by the difference in feed intake. This provided net energy determined by the feeding trial method.

Lignin was determined by the method of Ellis *et al.* (1946); nitrogen and ash were determined by A.O.A.C. procedures (1956). Holocellulose

TABLE 1. COMPOSITION AND DIGESTIBILITY DATA

Items	Chopped alfalfa	Pelleted alfalfa
Composition of alfalfa:		
As harvested: [a]		
Nitrogen, %	3.69	3.69
Lignin, %	7.3	7.3
As fed:		
Nitrogen, %	3.65	3.74
Lignin, %	7.4	7.2
Holocellulose, %	41.6	40.6
Digestibility:		
From total collection:		
Organic matter, %	64.2	65.1
Nitrogen, %	71.6	74.6[c]
Holocellulose, %	58.7	58.5
Lignin, %	−8.5	−4.4
From lignin ratio: [b]		
Organic matter, %	67.1	66.5
Nitrogen, %	73.7	75.7[c]
Holocellulose, %	61.9	60.2

[a] This sample was obtained before harvesting by taking plants at random throughout the field.
[b] All digestibilities calculated from the lignin ratio were significantly higher than those determined by the total collection technique.
[c] The nitrogen digestibility was significantly greater for the pelleted alfalfa than for the chopped alfalfa.

was determined as suggested by Wise *et al.* (1946). Previous difficulty in determining holocellulose in feces was found to be due to a variation in pH caused by an inadequate water extraction of the salts which acted as buffers. Statistical procedures are described by Snedecor (1946).

Results

The lignin, nitrogen and holocellulose content was essentially the same in the chopped as in the pelleted alfalfa (table 1). Furthermore, there was essentially no difference in the lignin and nitrogen content of the alfalfa before harvest and the alfalfa after dehydration in a commercial heated-air, drum-type drier.

Pelleted alfalfa did not differ from the chopped alfalfa in digestibility of organic matter, holocellulose or lignin. However, the nitrogen was apparently more digestible in the pelleted alfalfa. The digestibilities of organic matter, nitrogen and holocellulose were higher when calculated

TABLE 2. ENERGY CONTENT[a] OF THE HAYS

Item	Chopped alfalfa	Pelleted alfalfa
Total digestible nutrients, %	58.7	59.5
Gross energy, Kcal. per Kg.	4512	4451
Digestible energy, Kcal. per Kg.	2735	2795
Metabolizable energy, Kcal. per Kg.	2259	2295
Net energy, Kcal. per Kg.	1380	1433

[a] Dry matter basis.

by the lignin ratio method than when calculated by the total collection method. This was due to the negative lignin digestibilities. However, this does not negate the usefulness of the lignin ratio technique because the relative differences between chopped and pelleted alfalfa by either the total collection or lignin ratio technique would be similarly interpreted.

There was no significant difference between the energy content of chopped and pelleted alfalfa hay (table 2). This is in contrast, with the exception of net energy, to the results reported by Blaxter and Graham (1956). They reported a lower TDN, digestible and metabolizable energy in pelleted, ground grass hay. Earlier Forbes *et al.* (1925), from calorimetric work with steers, had reported that the net energy of chopped and ground alfalfa was equal. They did report a slightly lower digestible and metabolizable energy for the ground alfalfa. Their alfalfa was lower in energy content than that reported in this paper. Swift *et al.* (1950) reported digestible and metabolizable energy on an alfalfa which is in agreement with our results. Swanson and Herman (1952) have also reported that grinding alfalfa through a 5/16 inch screen did not lower digestibility. Direct comparisons cannot be made with the data of Long *et al.* (1955) because before pelleting the hay was hammered through a coarser screen (½ inch), concentrate was included in the ration, and chemical analyses were not given.

An inspection of the data from the growth study (table 3) in which

TABLE 3. GROWTH AND FEED DATA

	Maintenance		Rapid growth		
Item	Chopped hay	Controlled feed intake	Chopped hay *ad libitum*	Controlled feed intake	Pellets *ad libitum*
No. of lambs	6	6	6	6	6
No. days on experiment	56	56	56	56	56
Average initial wt., lb.	78	78	75	75	76
Average daily gain, lb.	—0.02	—0.01	0.27	0.26	**0.40
Daily feed consumed, lb.	1.66	1.67	2.48	2.54	3.25
Gain per 100 lb. feed, lb.	—	—	11.0	10.4	12.3
Carcass fat, %	29.5	31.2	30.8	30.8	28.4

** Highly significant difference from those on chopped hay.

the animals were pair-fed equal quantities of chopped or pelleted hay at two feeding levels reveals no difference in gain. Furthermore no significant difference in carcass fat was found which indicates little difference in the composition of the gain. The carcass fat was not different for the sheep fed at the maintenance level and those fed to gain rapidly. This was surprising, but earlier Garrett *et al.* (1958) also noted this for sheep but not for cattle when fed to gain at various rates.

The feed intake of the pair-fed sheep was always limited by the animal fed chopped hay. Generally 20 min. were needed for the pellets to be consumed while 90 min. were needed for consumption of the chopped hay. When pellets were fed *ad libitum,* feed consumption was greater and a greater gain resulted. These data indicate that increased gain of sheep fed a pelleted ground roughage is due to increased feed intake.

At the end of the trial the pair-fed sheep were killed at prescribed intervals after feeding and studies made on the digestive tract contents. The total and dry matter content of the reticulo-rumen was less for the pelleted hay fed sheep than for those fed chopped hay (figure 1). The smaller content of the reticulo-rumen of the pelleted hay fed animals was mainly due to lower holocellulose. No significant differences existed in total dry matter or holocellulose content of the remaining organs in the gastrointestinal tract. Therefore, this seems to indicate a faster passage of feed from the reticulo-rumen of the pelleted hay fed sheep rather than any difference further along in the tract. This agrees with Blaxter *et al.* (1956) that pelleted ground roughage passes through the digestive tract by virtue of a faster movement from the reticulo-rumen.

The lignin-holocellulose ratio indicates a faster breakdown of holocellulose by the rumen microorganisms of the sheep fed pelleted hay (figure 2). Less holocellulose per unit of lignin at 1½ and 4 hr. after feeding indicates a greater rate of holocellulose digestion. In this case the interaction of time and method of feeding was statistically significant.

The studies of the fatty acid production by rumen contents in the Warburg confirm that a faster rate of digestion occurs when hay is ground and pelleted (figure 3). A much greater fatty acid production from the rumen contents occurred at 1½ and 4 hr. after feeding for the pelleted hay fed animals as compared to chopped hay fed animals. Here the interaction of time after feeding and type of hay was highly significant. It should be pointed out that difficulty in obtaining representative rumen content by stomach tube occurred more often when chopped hay was fed. This may or may not have magnified the differences. Balch and Rowland (1957) also noticed a more rapid rate of fatty acid production when hay was finely ground.

The nitrogen content (figures 1 and 2) was greater at all times in the rumen of the pelleted hay fed sheep. Possibly the difference in nitrogen content would be due to a greater number of microorganisms. This coupled with the finer hay particles from the pellets which would allow more

surface for digestion might explain a greater rate of cellulose breakdown.

A visual examination of the rumen contents 1½ and 4 hr. after feeding revealed a more fluid type of contents for the pelleted hay fed sheep as compared to the chopped hay fed animals. Interestingly enough, no signi-

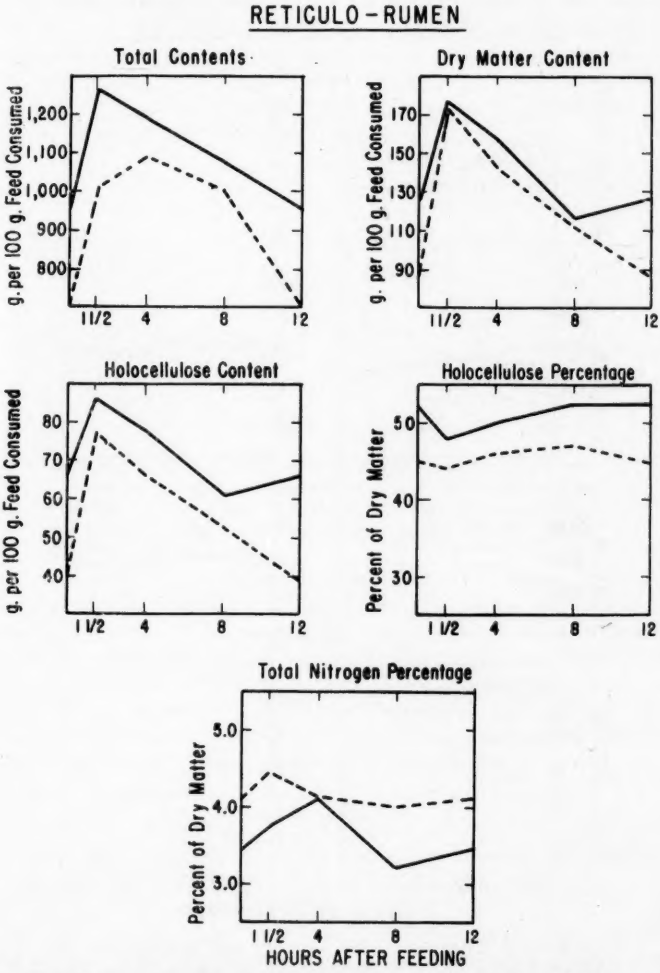

Figure 1. Observation on the reticulo-rumen content of sheep killed at prescribed times after feeding. The solid line represents the sheep fed chopped hay while the broken line represents the sheep fed pelleted hay.

ficant difference was found in the water percentages. The difference was due to the fineness of particles. As the time after feeding lengthened, the reticulo-rumen contents became more alike; due no doubt to the greater

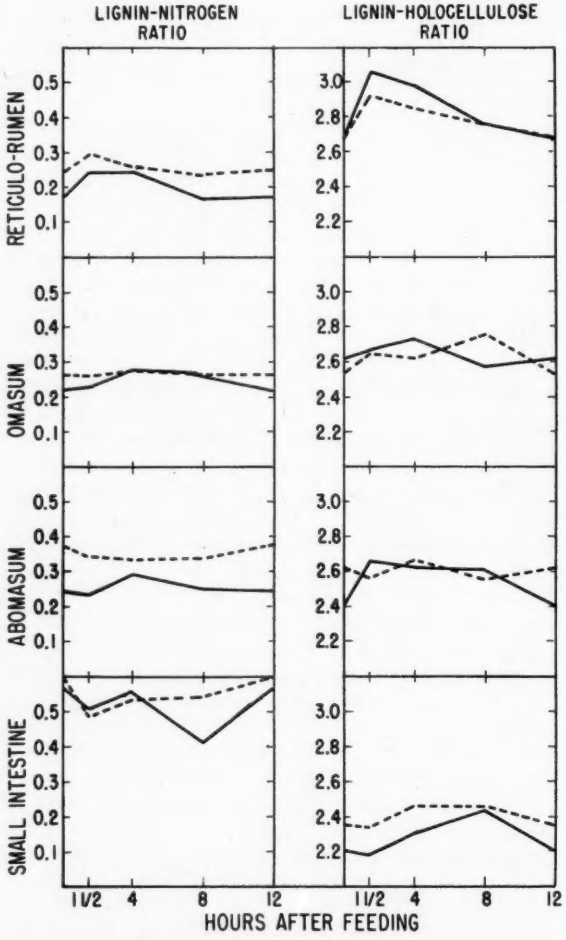

Figure 2. Changes in the nitrogen and holocellulose in different parts of the digestive tract as influenced by time after feeding and method of hay preparation. The solid line represents the sheep fed chopped hay while the broken line represents those fed pellets.

amount of remastication by the chopped hay fed sheep. It might be postulated that grinding the roughage before pelleting relieved the sheep from remastication—another factor in a faster rate of feed passage when ground hay is pelleted.

Lignin-nitrogen and lignin-cellulose ratios were the same in the omasums of the chopped and pelleted hay fed animals. For some unexplainable reason there was a highly significant difference in the lignin-nitrogen ratio of the abomasum. More nitrogen was present in the abomasum of the pelleted hay fed sheep. However, there was no detectable difference in the small intestinal lignin-nitrogen ratios.

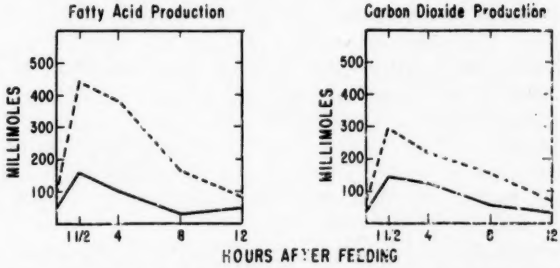

Figure 3. The in vitro fatty acid and carbon dioxide production of rumen contents from sheep killed at various intervals after feeding. The solid line represents the sheep fed chopped alfalfa hay while the broken line represents those fed pellets.

Carbon dioxide production (figure 3) by the rumen contents of the pelleted hay fed sheep was much greater at 1½ and 4 hr. after feeding than by the rumen contents of the chopped hay fed sheep. It was noted earlier that distention of the rumen was more apparent for the pellet fed sheep. However, serious bloating never did occur and the sheep fed pellets did not show any more signs of discomfort than those fed the chopped hay. No difference in methane production was observed.

Discussion

It is postulated that the increased feed intake resulting from grinding and pelleting hay is the direct result of a faster rate of digestion in the reticulo-rumen. This accelerated digestion allows a faster passage of feed through the digestive tract. The finer hay particle size, more logically, might be the reason for this. In this case pelleting reduces bulk but more important, from a feeding value standpoint, makes a fine dusty feed more palatable. The completeness of digestion, even though proceeding at a more rapid rate, should be the same for pellets and chopped hay because

lignification limits digestion to the same degree in both cases. However, it has not been definitely shown in this or other studies which is the more important influence; the fine grinding or decrease in bulk due to pelleting, or both. We do suggest that the influence of fine grinding should not be ignored in considering the influence of pelleting roughages on their feeding value.

The lignin-ratio technique allows some calculations which indicate that nitrogen from alfalfa does not proceed in an orderly manner through the digestive tract. Since the sheep were fed a constant amount of feed 12 hr. apart, and time after feeding did not influence the lignin-nitrogen ratios, one can calculate change in total nitrogen content throughout the tract. Of the 21.25 gm. of nitrogen fed per feeding only 49.5% passed the omasum. For both pelleted and chopped hay 50% of the nitrogen was apparently lost and probably absorbed as ammonia which would be of little value to the sheep. Annison (1956) found no evidence of amino acid absorption from the rumen. Our work confirms Gray and Pilgrims' (1956) observation that only 40% of the nitrogen of alfalfa reaches the abomasum whereas 100% of the nitrogen of wheaten hay reached the abomasum. Balch (1957) also reported a large loss of nitrogen from the rumen.

An increase in the nitrogen content occurred in the small intestine. From the lignin-nitrogen ratios it was calculated that 10.7 and 14.6 gm. of nitrogen passed through the abomasum but 21.4 and 22.6 gm. of nitrogen passed through the small intestine of the chopped and pelleted hay fed animals, respectively. However, only 5.3 and 5.4 gm. of nitrogen were excreted in the feces by these animals. This is about a 175% increase in nitrogen from enzyme and other secretions into the small intestine. This mixing of endogenous, microbial and food nitrogen could possibly be a large factor in the overall nitrogen and amino acid economy of the body. Dreisback *et al.* (1954) noted that an amount of nitrogenous material was recovered from the gastrointestinal tract of rats which was always equal to or greater than the amount of protein ingested. Also Boyne *et al.* (1956) found a very large addition of nitrogen to the small intestine of sheep. A study of the amino acids of intestinal contents led Nasset *et al.* (1955) to suggest that qualitative amino acid composition of intestinal contents is not greatly altered by changing from a non-protein to a protein containing test meal and that one of the amino acid supplements to a poor quality protein may be provided by the intestine itself; also, that the addition of nitrogen as digestive enzymes, muco-proteins and other nitrogenous compounds to feed protein results in a blending which changes considerably the nutritional value of the feed protein. Our study with sheep reveals that quantitatively a very large amount of nitrogen from endogenous sources is blended with feed and microbial protein. This suggests that this blending of nitrogen is a very large portion of the turnover of the labile nitrogen pool of the body.

The results for both the pelleted and chopped alfalfa hay indicate that 89% of the holocellulose was digested by the time it reached the abomasum. This is larger than the 60% reported by Gray (1947) but comparable to that found by Hale *et al.* (1947).

Summary

Sheep were pair-fed chopped and finely ground pelleted alfalfa hay. Two feeding levels were used. Studies were made on growth, feed intake, digestibility, energy utilization and content of the hay, *in vitro* carbon dioxide and fatty acid production by the rumen contents at various times after feeding, and changes in the ingesta as it passes through the digestive tract.

Digestibility of lignin and holocellulose was not greatly influenced by grinding and pelleting but nitrogen digestibility was higher for the pelleted hay. Eighty-nine percent of the holocellulose of both the chopped and pelleted hay was digested in the reticulo-rumen. Total digestible nutrient, digestible, metabolizable and net energy content were not significantly different between the pelleted and chopped hay.

The results indicate that increased gains due to feeding the finely ground, pelleted alfalfa hay compared to chopped alfalfa hay are due to an increased feed intake. A faster passage of ingesta from the reticulo-rumen seemed to be responsible for this increased feed intake. This in turn was due to a faster rate of holocellulose digestion by the rumen microorganisms as indicated by the lignin-holocellulose ratios and the *in vitro* fatty acid production by the rumen contents.

By using lignin-nitrogen ratios, calculations indicate that approximately 50% of the feed nitrogen was lost before the ingesta reached the omasum. A lowered nitrogen content persisted through the abomasum, but over a 175% increase in nitrogen was found in the small intestine. From these last observations, it is suggested that the additions of nitrogen as enzymes and other secretions which blend with the feed and microbial nitrogen in the small intestine have an important influence on the nitrogen utilization and economy of the body.

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THE EFFECT OF TWO LEVELS OF ENERGY INTAKE ON REPRODUCTIVE PHENOMENA IN DUROC JERSEY GILTS[1]

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SEVERAL studies have indicated that the amount of feed consumed by gilts has altered certain reproductive factors. Burger (1952) and Robertson *et al.* (1951) reported that restricted feeding delayed puberty, while Christian and Nofziger (1952), Self *et al.* (1955) and Sorensen and Gossett (1956) found that full-feeding delayed puberty. Self *et al.* (1955) and Zimmerman *et al.* (1957) increased ovulation rates by short periods of full-feeding, while Wise and Robertson (1953) decreased ovulation by limited-feeding after gilts reached 175 lb. in weight. However, Gossett and Sorensen (1956) found that gilts fed a ration containing either 50 or 75 therms of productive energy per 100 lb. of feed had the same ovulation rates. The results of all previous studies agree that embryonic mortality is higher in gilts that are full-fed than in gilts which are restricted in feed intake.

The present experiment was conducted to determine what effect a restriction of approximately 50% in energy intake would have on the age and weight at puberty and subsequent reproduction in Duroc-Jersey gilts fed on pasture. Data collected include the age and weight at puberty, ovulation rates at first and second heat, the degree of embryonic survival and related phenomena.

Materials and Methods

The Duroc gilts were produced at the Experiment Station Farm in the same farrowing season of two consecutive years. In July, 1954, 46 gilts were placed on experiment and during July, 1955, 56 gilts were used to replicate the first year's study. Two gilts died before they attained puberty and two died before complete ovulation data were obtained. In the first year the gilts averaged 114 lb. and 121 days of age when placed on test, and in the second year 130 lb. and 126 days of age.

Each year the gilts were divided into two groups of equal weight, age and ancestry and placed in pasture lots of similar size and forage content. Both groups were hand-fed twice daily. One group was designated as full-fed, and was fed all that they would consume of a 16% protein ration (table 1). The other group, designated as limited-fed, was fed one-half this quantity of a second ration (table 1). This ration contained approxi-

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mately the same amount of TDN, but approximately twice as much crude protein, minerals, vitamins and antibiotic. By this method of feeding it was hoped that the energy intake of the second group would be reduced to approximately 50% of the first group, while the intake of other essential nutrients would be similar for the two groups.

The gilts were weighed at 14-day intervals and weights used in data computations were obtained by interpolation. Daily estrous checks with vasectomized boars were initiated when the experiment began, to obtain age and weight at puberty. The gilts were checked during the first estrual cycle and bred on the first and second day of the second heat period.

TABLE 1. COMPOSITION OF TEST RATIONS (PERCENT)

Ingredients	Full-fed ration	Limited-fed ration
Ground corn	50.00	16.00
Ground oats	23.00	0.00
Alfalfa leaf meal	10.00	20.00
Soybean oil meal	10.00	44.00
Meat and bone scraps	5.00	17.00
Limestone	1.00	1.00
Steamed bone meal	1.00	1.00
Trace mineralized salt	0.53	1.06
Aurofac 2A (Lederle)	0.30	0.60

Approximately a third of the gilts in each ration group were selected as herd replacements and were allowed to farrow. The remaining two-thirds of the gilts were assigned alternately to be sacrificed at 3 or 25 days after breeding to obtain data on fertilization rate, embryonic survival and ovulation rate. The gilts that farrowed supplied data on number of live pigs farrowed and allowed an estimate of embryonic mortality between 25 days and term. There were 34 gilts in the 3-day group, 35 in the 25-day group and 29 that farrowed.

The data were subjected to analysis of variance tests as described by Snedecor (1946). Differences between years of study, ration treatments and stages of gestation were analyzed for statistical significance.

Results and Discussion

Age and Weight at Puberty. All gilts exhibited heat during the experimental period. The mean age at puberty for the 46 gilts in the first year was 206 days and for the 54 gilts in the second year it was 207 days. The range in age at puberty for all gilts in the study was between 168 and 276 days. Differences between years within ration treatments were not significant. The limited-fed gilts averaged 217 ± 17.1 days of age, while full-fed gilts were 195 ± 16.5 days at puberty. This difference was significant ($P < 0.01$). The full-fed gilts gained an average of 1.0 lb. per day while limited females

gained only 0.4 lb. per day up to breeding. A summary of these results is presented in table 2.

The fact that the limited-fed gilts averaged 22 days older at puberty than the full-fed gilts is in general agreement with results of Burger (1952) and Robertson *et al.* (1951). Burger, however, retarded the age at puberty by more severe feed restrictions than used in the present study, while Robertson found a smaller difference with less feed restriction. Studies by Christian and Nofziger (1952) and Self *et al.* (1955) do not agree with the results of this study. They found that restricted feeding caused gilts to reach puberty earlier than those receiving larger amounts of feed. The trial by Gossett and Sorensen (1956) showed that gilts on the lower energy ration reached puberty 21 days before those on the higher energy

TABLE 2. AVERAGE AGE AND WEIGHT OF GILTS AT PUBERTY, BY RATION TREATMENT AND YEAR OF STUDY

Ration group and year of study	Limited-fed			Full-fed		
	1	2	Combined	1	2	Combined
Number of gilts	23	28	51	23	26	49
Average age, days	216	219	217	196	195	195
Average weight, lb.	158	161	160	194	197	196

ration. Several reports have suggested that overly fat animals are slower to reach sexual maturity, which might explain why full-fed gilts in some experiments took longer to reach puberty than limited-fed gilts.

The weight at puberty for all gilts in the study ranged from 128 to 290 lb., with a mean of 178 lb. Differences between years of the study were not significant. There was an average difference of 36 lb. between the weights of limited and full-fed gilts at puberty. The mean weight for limited-fed gilts was 160 ± 18.8 lb. and for full-fed gilts 196 ± 26.7 lb. which was highly significant ($P < 0.01$).

The fact that full-fed gilts were heavier and reached puberty before limited-fed gilts indicates that size or growth rate might have affected the attainment of puberty. This theory is supported by work of Warnick *et al.* (1951), who found that heavier pigs within inbred lines tended to reach puberty before slower growing females. Also, McKenzie (1928) suggested that a low plane of nutrition retarded the growth of reproductive organs in gilts. Since feeding level influences the growth rate, it must also have an effect on gonadotrophin and ovarian activity which influences the attainment of puberty.

First Estrual Cycle Length and Fertilization Rate at Second Estrus. The average length of the first estrual cycle for all gilts was 20 days. All but five gilts had estrual cycles between 18 and 22 days. Limited-fed gilts had a mean cycle length of 19.7 days compared to 20.4 days for full-fed gilts. Dif-

ferences between years and ration treatments for estrual cycle length were both non-significant. The average first estrual cycle in this study was about one day shorter than reported by Robertson *et al.* (1951) and Self *et al.* (1955).

Approximately 80% of the ova, based on corpora lutea count, were recovered. The ova recovery rate in the present study was comparable to that reported by Hauser *et al.* (1952) and Robertson *et al.* (1951). Ninety-eight percent of the ova from limited-fed gilts and 95% from full-fed gilts showed various stages of cleavage. Since fertilization rates were so high and differences between ration treatments were not significant, it may be assumed that the percentage of fertilization was of minor importance in determining embryonic survival.

TABLE 3. AVERAGE OVULATION RATES AT FIRST AND SECOND HEAT RECORDED AT SLAUGHTER AT THREE DAYS POSTBREEDING ON SECOND HEAT

Ration group and year of study	Limited-fed			Full-fed		
	1	2	Combined	1	2	Combined
Number of gilts	8	10	18	7	9	16
First heat	10.3	9.6	9.9	13.7	12.0	12.8
Second heat	10.4	11.2	10.8	14.3	13.4	13.8
Increase (1st to 2nd)	0.1	1.6	0.9	0.6	1.4	1.0

Ovulation Rate at First and Second Heat. Ovulation rates for both the first and second heat periods were obtained from gilts sacrificed 3 days postbreeding (table 3). At first heat ovulation was slightly higher in the first year for both limited and full-fed gilts. Full-fed gilts ovulated an average of 3.4 more ova than limited-fed gilts in that year. At the second heat the difference was 2.4 eggs in favor of full-fed gilts. Data from both years combined showed that the full-fed gilts shed 2.9 more ova at first heat than limited-fed gilts. Ovulation rates ranged from six to nineteen ova at the first heat.

For the second heat period the difference was 3.0 in favor of the full-fed animals. The differences at both first and second estrus were significant ($P<0.01$). Ovulation rate ranged from five to fourteen for limited-fed gilts at second heat and from nine to nineteen for full-fed gilts. Differences between years in ovulation rates at either heat period were non-significant.

The difference in ovulation rates between ration treatments shows the same trend as reported by Christian and Nofziger (1952), Robertson *et al.* (1951) and Self *et al.* (1955). However, Gossett and Sorensen (1956) found about the same ovulation rate under two levels of energy intake. The increased ovulation from the first to second heat was apparently a normal phenomenon.

Although Lerner *et al.* (1957) and Rathnasabapathy *et al.* (1956) reported that approximately 0.5 more ova were shed for each 10 days increase in age at breeding, this evidently is not the case when levels of nutrition are altered. The limited-fed gilts were an average of 22 days older at each heat period and yet they ovulated from two to three less eggs per heat than full-fed gilts. Self *et al.* (1955) and Zimmerman *et al.* (1957) showed that gains made by gilts prior to breeding affected ovulation rate. This coincides with the trend in the present study, as full-fed gilts were the more rapid gainers and had the higher ovulation rates.

Ovulation Rate and Number of Embryos at 25 Days. Thirty-five gilts provided information on ovulation rate at second heat and the number of normal embryos at 25 days of gestation. The ovulation rates were very similar within ration treatments in the two years and differences were not significant (table 4).

TABLE 4. AVERAGE OVULATION RATE AND NUMBER OF EMBRYOS AT 25 DAYS OF GESTATION

Ration group and year of study	Limited-fed			Full-fed		
	1	2	Combined	1	2	Combined
Number of gilts	8	10	18	8	9	17
Ovulation rate	11.1	11.0	11.1	12.5	12.8	12.7
Normal embryos	9.3	10.4	9.9	10.1	10.1	10.1
Embryo loss	1.8	0.6	1.2	2.4	2.8	2.6

The ovulations ranged from eight to fifteen for limited-fed gilts and from seven to seventeen for full-fed gilts. The average difference of 1.6 ova more for full-fed gilts approached statistical significance at the 5% level. Left ovaries shed approximately one more ova than right ovaries ($P<0.01$). Similar findings in regard to differences in ovulation rate between left and right ovary have been reported by Lerner *et al.* (1957), Rathnasabapathy *et al.* (1956) and Squires *et al.* (1950). The ovulation rate for individual ovaries ranged from one to twelve.

The difference of 0.2 of an embryo between ration treatments at this stage of gestation was not significant. Differences were also non-significant between years for number of embryos. Although the full-fed gilts had slightly more embryos, this trend is in disagreement with studies by Robertson *et al.* (1951) and Self *et al.* (1955). They found that limited-fed gilts had significantly more embryos than full-fed gilts at 25 days of gestation. However, the limited-fed gilts in the studies by Robertson and Self received more feed in relation to their full-fed gilts than did the limited-fed gilts in the present study.

Size of Litters Farrowed. Data for litter size at term were provided by 29 gilts (table 5). By comparing litter sizes to an estimated ovulation rate,

the embryonic mortality between ovulation and term was determined. Litters ranged from four to thirteen live pigs for full-fed gilts and from seven to eleven for limited-fed gilts.

Differences in litter size due to either ration treatment or year of birth were not significant. Full-fed gilts farrowed a total of five dead pigs, compared to four for the limited-fed gilts. A total of eight mummified fetuses were farrowed; two by limited-fed gilts and six by full-fed gilts.

The difference of 0.3 of a live pig in favor of full-fed gilts was much smaller than the differences reported by Christian and Nofziger (1952) and Hanson *et al.* (1953), when gilts were restricted in feed intake. Hanson found that full-fed gilts farrowed 1.2 more pigs, but Christian

TABLE 5. AVERAGE NUMBER OF PIGS FARROWED AND EMBRYO LOSS

Ration group and year of study	Limited-fed			Full-fed		
	1	2	Combined	1	2	Combined
Number of gilts	7	7	14	8	7	15
Ovulation rate[a]	10.9	10.9	10.9	13.2	13.2	13.2
Number of live pigs	8.6	8.9	8.7	9.4	8.6	9.0
Embryo loss	2.3	2.0	2.2	3.8	4.6	4.2

[a] Estimation based on second heat ovulation of 3- and 25-day gilts.

reported that limited-fed gilts farrowed 2.7 more pigs than full-fed females. These differences may be attributed to the systems of feeding, management and breed effects. It must be concluded that limited feeding in the present study was not detrimental to litter size.

Prenatal Mortality. Prenatal mortality rates were determined at 25 days of gestation and at term. These calculations were based on complete fertilization of the ova shed, although fertilization was slightly less than 100%. The number of corpora lutea and normal embryos in 35 gilts sacrificed at 25 days after breeding provided data for the embryonic loss between ovulation and 25 days of pregnancy. The ovulation rate of 69 gilts at second heat was used as the "estimated" ovulation rate for the 29 gilts that farrowed for determining embryonic loss between ovulation and term. Losses between 25 days of gestation and term were determined by difference.

The prenatal mortality at 25 days of pregnancy ranged between 0 and 100%, with a mean of 11.6% for limited-fed gilts and 22.1% for full-fed gilts (table 6). In one case the number of embryos obtained was one more than the number of corpora lutea counted. Differences between years and between ration groups were non-significant, although the litters from full-fed gilts had twice the fetal mortality as those from limited-fed gilts.

The mean prenatal mortality rate between ovulation and farrowing was 26.1% and ranged from 0 to 69.7%. The limited-fed gilts lost 20.1% of ova, compared to 31.8% for full-fed gilts, between ovulation and farrowing (live pigs). The difference in embryonic mortality between the two rations

for the entire pregnancy was not as pronounced as that for the first 25 days of gestation. Differences in mortality rate due to rations and years were non-significant. The mortality rate was 25.2% and 26.9% in the first and second year, respectively.

Although a portion of the early embryonic loss may be attributed to the failure of ova fertilization, the data suggest that the first part of gestation is a critical period for survival of fertilized ova. This is in agreement with the findings of Burger (1952), Rathnasabapathy *et al.* (1956) and Robertson *et al.* (1951).

TABLE 6. PERCENTAGE OF EMBRYONIC MORTALITY BY RATION TREATMENT AND YEAR OF STUDY AT DIFFERENT PERIODS OF GESTATION

Ration group	Year of study	Ovulation to 25 days	25 days to term	Ovulation to term
Limited-fed	1st year	18.4	3.0	21.4
	2nd year	6.2	12.4	18.6
	Combined	11.6	8.5	20.1
Full-fed	1st year	22.1	6.8	28.9
	2nd year	22.2	12.8	35.0
	Combined	22.1	9.7	31.8
All litters	Both years	16.7	9.4	26.1

The findings in the present study that limited feeding is not as detrimental as full-feeding on embryonic survival agree with the trend indicated by Christion and Nofziger (1952), Robertson *et al.* (1951) and Self *et al.* (1955). However, the differences in embryonic mortality rates between ration groups were much larger in these other studies.

Summary

One hundred and two Duroc Jersey gilts were used to determine the effects of two levels of energy intake on attainment of puberty, ovulation rates and embryonic mortality. One-half of the gilts were limited to 50% of the energy intake of a full-fed group. All gilts were bred at second heat. One-third of each ration group was sacrificed at 3 days after breeding, another third 25 days postbreeding and the remainder allowed to farrow. Data were collected during the same season of two consecutive years.

The gilts on the limited-energy intake averaged 217 days of age and 160 lb. at puberty. Full-fed gilts averaged 195 days of age and 196 lb. at puberty. Differences between years in age and weight at puberty were extremely small.

Limited-fed gilts ovulated an average of 9.9 ova at first heat, compared to 12.8 ova by full-fed gilts. These same gilts had mean ovulation rates of 10.8 and 13.8 in the second heat. The fertilization rate of all ova recovered was 96.5%.

The mean ovulation rate at second heat for gilts sacrificed at 25 days of gestation was 11.1 and 12.7 ova for limited and full-fed gilts, respectively. There was a mean of 9.9 normal embryos for limited-fed gilts, and 10.1 for full-fed gilts at this state of pregnancy. Limited-fed gilts farrowed an average of 8.7 live pigs, compared to 9.0 live pigs for full-fed gilts. Differences between years of birth were not significant.

Prenatal mortality for the first 25 days of gestation was 11.6% for limited-fed gilts and 22.1% for full-fed gilts. Mortality during the entire gestation period was 20.1% for limited-fed gilts and 31.8% for full-fed gilts.

The results of this study indicate that restricting the energy intake delayed puberty, depressed ovulation rate and decreased embryonic mortality. Therefore, it appears that gilts should be full-fed for high ovulation rates, but limited-fed to attain higher embryonic survival rates.

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THE GONADOTROPHIC CONTENT OF PITUITARY GLANDS FROM GILTS AT TWO STAGES OF EARLY PREGNANCY AND ON TWO LEVELS OF ENERGY INTAKE [1]

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THE pituitary gland is one of the most important endocrine glands of the body, and is known to contain gonadotrophin activity. Brody and Kibler (1941) reported a correlation between weight of the pituitary gland and body weight of mature mammals. Baird *et al.* (1952), Elijah and Turner (1942) and Robinson and Nalbandov (1951) have all reported pituitary gland weights for various classifications of swine. Schultze and Turner (1948) and others agree, however, that the size of the gland does not indicate necessarily the degree of its activity or the amount of hormone stored in the gland.

Work by Nalbandov and Baum (1948) showed that Follicle Stimulating Hormone administration caused increased growth of the testes of male chickens due to the enlargement of the seminiferous tubules. The use of Luteinizing Hormone alone produced an increase in testes size by increasing the interstitial tissue. Baker *et al.* (1956), Hollandbeck *et al.* (1956) and Robinson and Nalbandov (1951) have used immature male chicks to study the gonadotropic hormone content of swine pituitary glands. The present study was conducted to compare the total gonadotropic content of pituitary glands from gilts fed two levels of energy and sacrificed at either 3 or 25 days after breeding.

Materials and Methods

The rations fed and general management were given in the previous paper by Haines *et al.* (1958). The study was conducted in the same season of two continuous years and all gilts were of similar breeding and age. Breeding occurred at the second estrous period. Twenty-eight of the gilts were sacrificed 3 days after breeding and the other 29 gilts 25 days after breeding.

The pituitary glands were collected within 30 minutes after sacrifice and the fresh weight of the entire gland recorded. The pituitary gland was then placed in a glass vial and stored in a frozen state at 15° F. until time of gonadotropic assay. The pituitary glands collected then were assayed, using immature White Leghorn male chicks in three separate test periods. Uniform technique and management was maintained during the three assay periods. The chicks were received the day of hatching

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from a private hatchery and were kept in standard chick brooders and fed a commercial starter ration.

At the time of assay the pituitary glands were allowed to thaw and the anterior lobe separated from the posterior lobe, stalk and connective tissue. The wet anterior lobe was then weighed, placed between two 3 x 4 inch glass plates and crushed with manual pressure. The glass plates, with the adhering tissue, were pulled apart, labeled and immediately placed in a calcium chloride desiccator. The plates remained in the desiccator for 24 hours at room temperature. The desicated gland material was scraped from the plates with a razor blade and weighed. The dry powder was then placed in a test tube and dissolved in distilled water. Two cc. of distilled water for each chick was added to the powder of a particular pituitary gland so that each gland was assayed individually. Between 3 and 4 mg. of powder was used for each chick and an effort was made to keep the amount of powder close to the 3-mg. level.

The chicks were 2 days old at the time of the first injection and weighed between 36 and 43 gm. Eight injections were given during a 4-day test period. At each injection, a chick received 0.25 cc. of the pituitary extract. Injections were administered subcutaneously either under the wing or on the back of the chick with a 22 gauge hypodermic needle. Chicks receiving no injection, as well as some injected with a commercial unfractionated sheep anterior pituitary gonadotrophin,[3] were included in each test group. A total of 8,000 I.U. (4 rat units) of chorionic gonadotrophin of the commercial material was injected per chick over the 4-day period.

At 6 days of age, or approximately 20 hours after the last injection, the chicks were killed and weighed. The two testes of each chick were removed, cleaned of adhering tissue and weighed. From two to seven chicks were used for the assay of each pituitary gland, depending upon the yield of the dry pituitary powder.

The average combined testes weights from chicks injected with an individual pituitary extract was used as a measure of the gonadotropic activity of the gland from a particular gilt. The difference in the average testes weights of the non-injected chicks and those injected with the commercial gonadotrophin was used to establish the measure of response to an International Unit of gonadotrophin. The gonadotrophin response in 3 mg. of dry pituitary powder was estimated by comparing with the response from the 8,000 I.U. of commercial gonadotrophin. Differences in the average testes weight between the chick assay groups were tested for significance by the analysis of variance as described by Snedecor (1946).

Results and Discussion

Weights of Pituitary Gland and Desiccated Pituitary Powder. The whole fresh pituitary gland weight from all gilts killed at 3 days post-

[3] Vetrophin, Abbott Laboratories.

TABLE 1. AVERAGE WEIGHTS OF WHOLE PITUITARY GLAND, ANTERIOR LOBE AND DESICCATED ANTERIOR LOBE BY GILT GROUP [a]

Gestation period	3-Day sacrifice		25-Day sacrifice		
Ration group	Limited	full	Limited	full	Overall means
Number of gilts	12	16	15	14	57
Whole gland	213.8	248.8	211.1	240.1	228.8
Anterior lobe	110.1	132.4	104.5	128.7	119.6
Desiccated anterior lobe powder	12.5	16.3	11.6	14.7	13.9

[a] All weights in mg.

breeding averaged 231 mg., while those from the gilts sacrificed at 25 days of gestation averaged 226 mg. The pituitary glands of full-fed gilts were about 30 mg. heavier in both stages of gestation than those of the corresponding limited-fed gilts. This average weight of 228.8 gm. (table 1) is similar to those reported by Elijah and Turner (1942) and Robinson and Nalbandov (1951), but higher than the 113.2 mg. found by Baird *et al.* (1952).

The 119.6-mg. average wet weight of the anterior lobe is similar to the 108- and 82-mg. reported by Baird *et al.* (1952), but less than the 219-mg. stated by Elijah and Turner (1942).

The 13.9-mg. average weight of the anterior pituitary powder that was obtained from the gilts in this study was considerably less than the 30-mg. obtained by Robinson and Nalbandov (1951) and the 39-mg. obtained from 225-lb. gilts by Baker *et al.* (1956) and Hollandbeck *et al.* (1956). Possibly the moisture content of the gland or powder was not the same in all studies, since the weights of the whole pituitaries have been similar in all studies.

TABLE 2. AVERAGE CHICK TESTES WEIGHT FOR ASSAY GROUPS AND THE ESTIMATED GONADOTROPHIC CONTENT OF THREE MILLIGRAMS OF INTERIOR PITUITARY POWDER

Assay test group	Number of gilts	Number of chicks	Average testes wt., mg.	Gonadotrophic contents, I.U.
Control chicks	—	30	14.9	——
Unfractionated sheep pituitary [a]	—	11	42.0	8,000
Stage: 3-day	28	115	15.7	236
25-day	29	110	21.5	1,948
Ration: Full	30	134	18.5	1,063
Limited	27	91	18.8	1,151

[a] Vetrophin, Abbott Laboratory.

Gonadotropic Assay Results. At the time of sacrifice the chicks weighed between 30 and 71 gm. and averaged 52.2 gm. The combined weight of both testes from all chicks averaged 18.7 ± 5.0 mg. and varied from 8.0 to 60.0 mg. per chick. The testes weights of all chicks in a particular assay group were averaged for treatment comparisons. The average chick testes weight for the different assay groups and the estimated International Units of total gonadotrophin in 3 mg. of pituitary powder is shown in table 2. By using growth stimulation from the commercial gonadotrophin as a standard, the total gonadotrophic content of the pituitaries was estimated. The testes weights of chicks used to determine gonadotrophin of pituitaries of three-day gilts averaged only 0.8 mg. heavier than those of the control chicks. However, the chick testes weight of the 25-day gilt group averaged 6.6 mg. heavier than those of the control chicks. Since approximately 3 mg. of pituitary powder was used per chick, the total

TABLE 3. SUMMARY OF ANALYSIS OF VARIANCE FOR AVERAGE CHICK TESTES WEIGHT DIFFERENCES IN THE GONADOTROPHIC ASSAY

Source of variance	D/F	Mean squares
Chick test groups	3	481.96**
3-day vs. control	1	0.59
3-day + control vs. 25-day	1	82.10**
25-day vs. commercial gonadotrophin	1	620.78**
Trial periods	2	13.43
Within sub-groups	6	4.40

** $P<0.01$.

gonadotrophin content of an entire anterior pituitary lobe, yielding an average of 13.9 mg. of powder, would be 1,110.3 I.U. for three-day gilts, and 9,020.6 I.U. for the 25-day gilts.

Significance of Testes Weight Differences. There was no statistically significant difference in gonadotrophic content of the pituitaries from gilts on the two ration treatments. The average chick testes weights were 18.8 mg. for the limited-fed gilts and 18.5 mg. for the full-fed gilt group. The average testes weights of 14.9 mg. for the control chicks in this study was comparable to the 16.0-mg. weight reported by Ullrey *et al.* (1955), but heavier than the 9.3-mg. found by Robinson and Nalbandov (1951). The fact that the gonadotrophic content was high in the 25-day-gilt group confirms the findings of Hollandbeck *et al.* (1956).

The results of an analysis of variance test on these differences in testes weights is summarized in table 3. The statistical analysis of the data indicates that there is a non-significant difference between the three-day gilt group and the control group. However, the testes of chicks in the 25-day gilt group were significantly heavier than those of either the con-

trol or three-day groups. Also, the testes of the chicks injected with commercial gonadotrophin were significantly heavier than those of any chick assay group.

Principles of Gonadotrophic Assays. Although in other studies 10 mg. of pituitary powder per chick have been used for a gonadotrophin assay, these authors showed that approximately 3 mg. was satisfactory for demonstrating differences in stage of gestation. The difference in dosage used could indicate that this powdered material in this study was more concentrated. It might be assumed, however, that the testes response to greater concentrations of pituitary extract would have been larger and, therefore, estimated values of total gonadotrophin more precise if 10 mg. had been used in place of the 3 mg.

Robinson and Nalbandov (1951) suggest that the gonadotrophin assay indicates the rate at which the hormone is being secreted when the animal is sacrificed. The method used in the present experiment does probably determine the total gonadotrophic content of the pituitary at time of sacrifice, but whether this total activity is classified as stored hormonal material or material about to be secreted by the pituitary gland is not established definitely.

Summary

Fifty-seven Duroc gilts were sacrificed at either 3 or 25 days after breeding and their pituitary glands obtained. The gilts had all been bred during their second estrous period. One-half of the gilts were given a full-feed ration while the others were limited-fed approximately 50% of the energy intake of the full group from a weight of 115 lb. to the time of sacrifice. The total gonadotrophic content of the anterior pituitary gland was assayed by the use of immature male chicks.

The gonadotrophic content of pituitaries from gilts sacrificed at 3 days postbreeding was not sufficient to produce significant testes growth response above control chicks. The pituitary glands from gilts pregnant for 25 days possessed a significantly greater gonadotrophic content than those from gilts killed 3 days postbreeding. A commercial gonadotrophin containing 8,000 I.U. of chorionic gonadotrophin stimulated testes growth significantly more than pituitary material from gilts of 25-day pregnancy. The gonadotrophic content of 3 mg. of pituitary powder from gilts killed at 3 and 25-days postbreeding was estimated to be 236 and 1,948 I.U., respectively.

The ration treatment to which gilts were subjected did not affect the gonadotrophin content of pituitary glands. The results also indicated that 3 mg. of anterior pituitary lobe powder per chick was sufficient for comparing gonadotrophin response due to stage differences.

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GROWTH POTENTIAL OF SWINE AS MEASURED BY SERUM ALKALINE PHOSPHATASE [1]

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PREVIOUS reports have shown that serum alkaline phosphatase activity reflects various pathological conditions (Kay 1930), nutritional states (Correll and Wise, 1938; Hibbs *et al.*, 1945; Combs *et al.*, 1955), and that the concentration of this enzyme declines with age in several species of animals (Bodansky, 1934; Young and Underdahl, 1948; Earle, 1952).

The relationship found to exist between phosphatase activity and growth in dogs (Bodansky, 1934), foals (Earle, 1952), and cattle (Kunkle *et al.*, 1953; Fletcher *et al.*, 1956), coupled with the desirability of obtaining an efficient criterion for predicting the performance of potential market swine, prompted the initiation of this study.

Experimental

A total of 321 baby pigs were used in this study. The purebred breeds included Duroc, Hampshire, and Spotted Poland China (SPC). Crossbred Hampshire x SPC pigs were also used. For approximately 7 days post-farrowing, dams and litters were kept in the farrowing stalls and then placed on alfalfa-clover pastures. All pigs had access to a creep ration from the time they were two weeks of age until weaning at 56 days.

Serum alkaline phosphatase activity was determined by the method of Bessey *et al.* (1946), in which 0.1 ml. of serum is used and activity is expressed in millimol (mM) units. The blood samples were obtained by heart puncture, when the pigs were 1 and 7 days of age, and centrifuged immediately. The sera were then frozen until analyzed.

Results and Discussion

The mean values and variance summary for birth weight, phosphatase activity at 1 and 7 days of age and 56-day weaning weight are presented in table 1. The within sex correlation coefficients for the various factors studied are shown in table 2. The birth weight of male pigs in all groups studied was significantly higher than that of females. This is in agreement with work of McKenzie (1928) in which he reported that male pigs averaged 0.9 lb. heavier at birth than female pigs. In the Duroc

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TABLE 1. MEAN VALUES AND VARIANCE SUMMARY

Breed	No. of animals	Birth wt., lb.	1-Day phosphatase, mM	7-Day phosphatase, mM	Weaning wt., lb.
Duroc					
Male	95	2.90	5.98	4.50	31.18
Female	100	2.64	6.47	4.94	30.00
Combined	195	2.76	6.23	4.73	30.57
SPC					
Male	5	3.62	4.08	3.98	46.40
Female	5	3.14	4.32	4.16	37.20
Combined	10	3.38	4.20	4.16	41.80
Crossbred					
Male	34	3.29	5.99	3.94	41.50
Female	41	3.19	6.39	3.82	44.22
Combined	75	3.24	6.21	3.87	42.99
Hampshire					
Male	17	3.07	5.52	3.06	28.24
Female	24	3.02	6.12	3.20	28.54
Combined	41	3.04	5.87	3.14	28.42

Items	d.f.	Birth wt. M.S.	1-Day phosphatase M.S.	7-Day phosphatase M.S.	Weaning wt. M.S.
Sex	1	.0990**	.3741*	.0841	6.76
Breed	3	.1398**	1.8312**	.8748**	111.90**
Sex x breed	3	.0187	.0116	.0315	13.33**
Within subgroup [a]	13	.0112	.0931	.0862	2.2864

[a] k=2.3572.
* P<0.05.
** P<0.01.

TABLE 2. WITHIN SEX CORRELATION COEFFICIENTS

Items	No. pigs	X_1Y	X_2Y	X_3Y
Duroc	195	.203**	.076	—.039
S.P.C.	10	.739*	—.678	—.344
X-Bred	75	.488**	—.067	.126
Hampshire	41	.517**	—.007	.092
Total, all breeds	326	.314**	.018	.006

X_1=Birth weight.
X_2 and X_3=Phosphatase activity at 1 and 7 days of age, respectively.
Y=56-day weight.
* Significant at 0.05 level.
** Significant at 0.01 level.

and SPC breeds this initial weight advantage was retained at weaning, but was lost in the Hampshire and Crossbred pigs. Thus, the over-all result was that sex had no significant effect on weaning weight. The r values obtained show that in all breeds birth weight was correlated significantly with weaning weight, the Durocs and SPC pigs, respectively, showing the lowest and highest degree of correlation. When all breeds were combined the r value was relatively small but significant. These data confirm the results of Blunn *et al.* (1954) who found a positive relationship between birth weight and 56-day weight ($r=0.53$) and who felt that birth weight is a relatively poor indication of future weights.

Both sex and breed exerted a significant effect on the phosphatase activity of the day-old pigs. The females in all breeds exhibited a higher activity than the males. When the pigs were 7 days of age, their phosphatase activity was considerably lower than the initial activity; and breed, but not sex, still exerted a significant influence. A similar situation regarding the effects that sex and breed have on phosphatase activity was found to exist with cattle (Kunkle *et al.*, 1953; Fletcher *et al.*, 1956). The correlation coefficients of both 1- and 7-day phosphatase activity and weaning weight were quite variable, r values ranged from -0.68 to $+0.13$. However, none of these coefficients was significant, which tends to eliminate the possibility of using the phosphatase activity of 1 and 7-day-old pigs as an index for predicting 56-day weaning weight.

Summary

The phosphatase activity of 321 pigs was determined when they were 1 and 7 days of age. Both birth weight and phosphatase activities were correlated with weaning weight at 56 days. A significant, but small, correlation coefficient ($r=0.31$) was found between birth weight and weaning weight. Male pigs weighed significantly more than female pigs at birth, but there were no significant sex differences in 56-day weaning weights.

Phosphatase activity at either 1 or 7 days of age was not significantly correlated with weaning weight. At 1 day of age female pigs exhibited a significantly higher phosphatase activity than male pigs. No sex differences were found in the 7th day determinations.

A significant difference among breeds was found with all criteria studied.

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LIGNIN AND METHOXYL GROUPS AS RELATED TO THE DECREASED DIGESTIBILITY OF MATURE FORAGES [1, 2]

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THE low digestibility of lignin by ruminants and the increased lignin content associated with advancing maturity serve as a basis for the assumption that lignification is one of the major factors responsible for the poor feeding value of mature forage plants.

In line with this general assumption, Forbes and Garrigus (1948; 1950a, b) were able to report significant correlations between the lignin content and the dry matter and organic matter digestibility and total digestible nutrients of various forages grazed by, or cut and fed green to, steers and wethers. Kamstra *et al.* (1958) demonstrated that the digestibility *in vitro* of the cellulose of plant materials cut at different stages of maturity decreased as the lignin content of the forages increased. Furthermore, it was shown that removal of lignin by the isolation of holocellulose resulted in greatly improved cellulose digestibility while the effect of the stage of maturity of the original plant material was greatly diminished.

In a recent study, Quicke *et al.* (1958) observed that the digestibility of the cellulose in mature brome and orchard grass hays differed considerably although the proximate composition and the lignin content of the different hay samples were very similar. These observations prompted a more detailed study of the lignin content of the hays and of four timothy hays which had been cut at different stages of maturity and which gave *in vitro* cellulose digestibilities which ranged from 83% to 42%.

The results of this work are reported in this paper and indicate that differences in the digestibility of the cellulose in mature forage samples cut at different stages of maturity cannot be explained solely in terms of lignin content or methoxyl groups.

Experimental

The forages used in this investigation are described in table 1.

The *in vitro* digestibility of the cellulose in each of the hays was determined by the technique described by Quicke *et al.* (1958) using resuspended ruminal microorganisms as inoculum.

Lignin was originally determined by the method of Crampton and Maynard (1938) as modified by Patton (1943). In an attempt to gain

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further information regarding the lignin present in the forages, "acid-insoluble lignin" and "total lignin" were determined according to the methods described by Moon and Abou-Raya (1952a, b). In carrying out the latter determinations, final hydrolysis with 3% H_2SO_4 was omitted but the precaution was taken to carry out all drying procedures *in vacuo* at a temperature of 60° C.

In order to obtain sufficient material for nitrogen and methoxyl group determinations, lignin determinations were carried out on triplicate samples, and tared sintered glass crucibles of coarse porosity were used for each filtration.

TABLE 1. DESCRIPTION OF FORAGES

Hay	Date cut (1957)	Description
Brome grass, 1st stage	June 10	Early bloom
Brome grass, 2nd stage	June 26	Seed stage: seed mature
Orchard grass, 1st stage	June 6	Early bloom
Orchard grass, 2nd stage	June 18	Early seed stage
Timothy, 1st stage	May 24	Vegetative stage
Timothy, 2nd stage	June 7	Heading
Timothy, 3rd stage	June 20	Headed
Timothy, 4th stage	July 16	Seed ripe

In carrying out the "total lignin" determination, it was found that centrifugation of the 72% H_2SO_4 digest (in 250 ml. centrifuge bottles) facilitated subsequent filtrations. After the final drying, crucibles and contents were weighed and the weight of crude lignin calculated. The crude lignin residues were then removed from the crucibles and the residues from the triplicate samples of a given forage were mixed and finely ground in an agate mortar. The composite lignin samples were dried *in vacuo* at 60° C. and stored in stoppered vials in a desiccator.

The ash content of the crude lignin was determined on duplicate samples by incineration at 650° C. and the nitrogen content was determined by a semi-micro Kjeldahl method using HgO as catalyst in the digestion procedure. Methoxyl groups were determined on duplicate samples (0.02g.) according to the TAPPI (1945) procedure.

In the determination of "acid-insoluble lignin" the 72% H_2SO_4 digest (undiluted) filtered extremely slowly. However, addition of 0.1 gm. Hyflo Super-cel (previously ashed at 650° C.) to the digest prior to filtration, reduced filtration time to 5 or 10 minutes. In one experiment, addition of 0.1, 0.2 and 0.3 gm. Super-cel to the digest did not result in any significant increase in the amount of ash-free lignin isolated from triplicate samples of orchard grass hay. This was taken as evidence that the Super-cel did not adsorb any of the breakdown products formed during the sulphuric acid digestion.

As in the case of the "total lignin" determination, crucibles and contents were weighed after the final drying step and the weight of the isolated

TABLE 2. PROXIMATE COMPOSITION AND CELLULOSE CONTENT OF BROME AND ORCHARD GRASS HAYS

Hay[a]	Percentage composition of dry matter					
	Ash	Protein	Ether extract	Crude fiber	NFE	Cellulose
Brome grass I	5.79	11.26	1.63	37.38	43.94	37.51
Brome grass II	5.65	10.68	1.66	35.94	46.07	35.38
Orchard grass I	9.58	11.45	2.59	35.67	40.71	37.47
Orchard grass II	9.07	11.46	2.02	36.86	40.59	38.04

[a] I and II refer to first and second stages.

lignin plus Super-cel was calculated. As previously described, lignin residues from triplicate hay samples were combined to give a composite lignin preparation, dried *in vacuo*, and analyzed for ash and nitrogen.

All lignin determinations were corrected for ash and the nitrogen and methoxyl contents of the lignin preparations were expressed on an ash-free lignin basis.

The methoxyl groups present in the original plant material were determined on 0.1 gm. oven-dried (*in vacuo*, 60°C.) samples using the TAPPI (1945) method. The proximate composition of the orchard and brome grass hays was determined by the standard A.O.A.C. procedures. Cellulose was determined according to the method of Crampton and Maynard (1938).

Results and Discussion

The analytical data are presented in tables 2, 3, and 4. All results are reported on a dry-matter basis. In view of the lack of agreement among different workers concerning the correction of lignin values for protein (NX6.25) content, total and acid-insoluble lignin values are reported as

TABLE 3. CELLULOSE DIGESTIBILITY AND LIGNIN CONTENT OF FORAGES CUT AT DIFFERENT STAGES OF MATURITY

Type of hay	Stage	Cellulose digestibility[a] %	Patton lignin ash-free %	Moon and Abou-Raya lignin				
				Total lignin		Acid-insoluble lignin		
				ash-free %	N-corrected %	ash-free %	N-corrected %	% of total lignin[b]
Brome grass	1st	52.9	16.38	17.48	14.58	10.55	8.56	60.35
	2nd	43.1	16.38	19.39	16.08	11.90	9.27	61.37
Orchard grass	1st	66.3	14.55	17.78	14.43	9.82	7.15	55.23
	2nd	53.3	16.68	19.95	16.50	11.40	8.61	57.14
Timothy	1st[c]	83.5	7.88	9.17	7.34	3.64	1.99	39.69
	2nd	75.8	11.07	13.09	10.96	5.80	5.22	44.31
	3rd	64.9	14.94	16.10	13.81	7.81	6.78	48.51
	4th	42.4	16.15	16.57	14.66	10.06	8.39	60.71

[a] Determined in 48-hour *in vitro* fermentation; average of 13 experiments.
[b] Calculations based on ash-free lignin.
[c] Cellulose content of timothy hays, % of dry matter: 1st, 27.9; 2nd, 32.4; 3rd, 34.1; 4th, 31.9.

both ash-free and N-corrected lignin. No correction was made for nitrogen in the case of the lignin values obtained by Patton's method as no provision was made for the collection of lignin samples for N analysis when these determinations were originally carried out.

In connection with the lignin values, it is of interest to note that while the values obtained by the Patton method are high in comparison with those obtained by the Moon and Abou-Raya method for acid-insoluble lignin, they are in close agreement with the total lignin values. Moon and Abou-Raya have shown (1952b) that values obtained by the method of

TABLE 4. TOTAL METHOXYL AND LIGNIN METHOXYL GROUPS IN FORAGES CUT AT DIFFERENT STAGES OF MATURITY

			Methoxyl groups of total lignin			Non-lignin methoxyl
Type of hay	Stage	Total methoxyl groups %	% of ash-free lignin	% of N-corrected lignin	% of forage dry matter	% of forage dry matter
Brome grass	1st	2.55	10.03	12.03	1.75	0.80
	2nd	2.82	9.79	11.81	1.90	0.92
Orchard grass	1st	1.68	6.83	8.42	1.21	0.47
	2nd	2.05	7.66	9.26	1.53	0.52
Timothy	1st	1.48	8.10	10.11	0.74	0.74
	2nd	1.94	9.45	11.28	1.24	0.70
	3rd	2.39	10.70	12.47	1.72	0.67
	4th	2.58	11.62	13.13	1.93	0.65

Ellis, Matrone & Maynard (1946), commonly used in the United States, are similar to their acid-insoluble lignin values but considerably lower than total lignin values. It appears therefore, that criticisms of Patton lignin values on the grounds that they are too high are open to question.

Data concerning the 48-hr. *in vitro* digestibility of the cellulose in the various hays are included in table 3. The validity of such data as a measure of the digestibility of cellulose in grasses has been discussed in a previous paper (Quicke *et al.*, 1958). As can be seen from the table, maturation of the various grasses resulted in a marked decrease in the digestibility of the cellulose present in the hays. In the case of first and second stage brome grass the cellulose digestibility dropped from 53% to 43% while the corresponding figures for orchard grass were 66% and 53%. In contrast to these noticeable differences in cellulose digestibility, these hays are very similar in respect of proximate composition, cellulose content, and lignin content as measured by Patton's method or by either of the Moon and Abou-Raya methods.

One point which warrants attention, however, is the fact that both samples of orchard grass show a higher ash content (about 9.3%) than the samples of brome grass (ash content about 5.7%). Since the *in vitro* cellulose digestibility of the former hays was noticeably better than that of the latter, it may be asked whether this observation can be explained in terms of the difference in the ash content of these two types

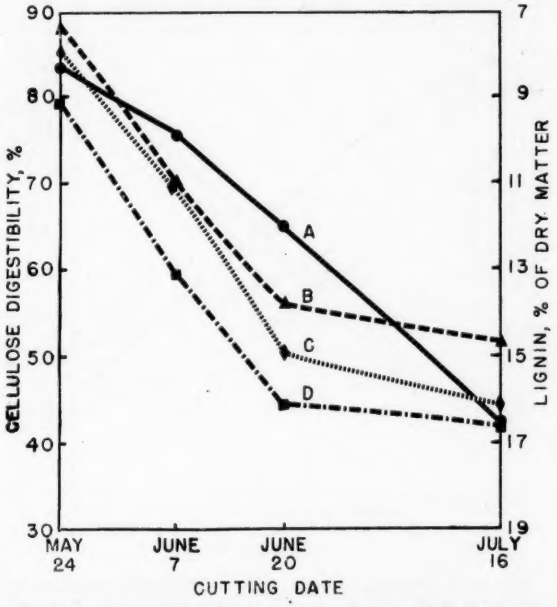

Figure 1. Changes in the in vitro cellulose digestibility and lignin content of timothy hay with advancing maturity. (A) In vitro cellulose digestibility; (B) N-corrected "total" lignin; (C) Patton lignin; (D) Ash-free "total" lignin.

of hay. This aspect has not been the subject of direct investigation but it should be stressed that a mineral mixture containing Na, K, Ca, Mg and Fe as their phosphates, chlorides or sulphates, depending upon the element in question, was used in the *in vitro* fermentation mixture (see Quicke *et al.*, 1958). The levels of the various mineral elements provided, compared favorably with the levels of these elements found in rumen juice (unpublished data) and it therefore appears unlikely that the lower cellulose digestibility observed with the brome hays can be explained in terms of the lower ash content of these hays.

Whereas both stages of brome grass and of orchard grass represented relatively mature forages it was deemed advisable to extend the study to a series of forages which would include material cut during the vegetative stage and at gradually advancing stages of maturity. Four stages of timothy hay (cf. table 1), all cut from a single pure stand, were selected for this purpose. As can be seen from the data in table 3 and from figure 1,

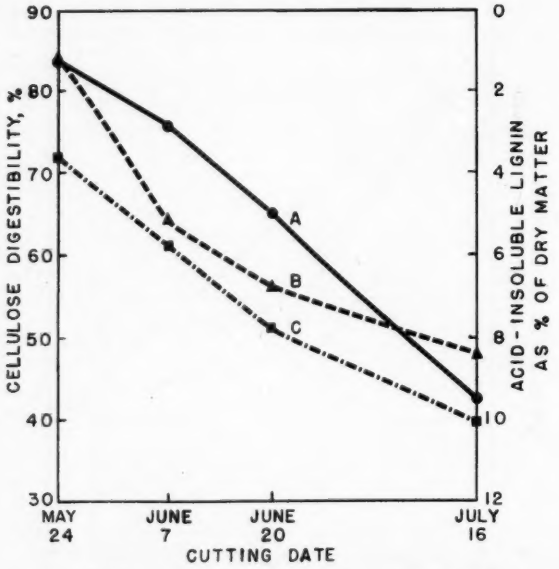

Figure 2. Changes in the in vitro cellulose digestibility and "acid-insoluble" lignin content of timothy hay with advancing maturity. (A) In vitro cellulose digestibility; (B) N-corrected "acid-insoluble" lignin; (C) Ash-free "acid-insoluble" lignin.

the decrease in the digestibility of the cellulose associated with advancing maturity is closely paralleled by the increase in Patton lignin and in "total" lignin up to the third stage of cutting. However, the subsequent increase in lignin content is almost negligible in comparison with the drop in cellulose digestibility (from 65% to 42%). Changes in total methoxyl and total lignin methoxyl follow very similar patterns.

On the other hand, the percent acid-insoluble lignin continued to increase appreciably after the third stage (figure 2) although, even in this case, the increase in lignin content does not continue at the same rate as does the fall in cellulose digestibility.

When the acid-insoluble lignin is expressed as a percentage of total lignin (cf. last column of table 3) it becomes evident that, as the timothy matured, an increasing proportion of the lignin was recovered in the acid-insoluble fraction. It appears, therefore that the nature of the lignin in the plant material gradually changes with advancing maturity, a conclusion which is substantiated by the increased methoxyl content of the total lignin isolated from the more mature timothy hays.

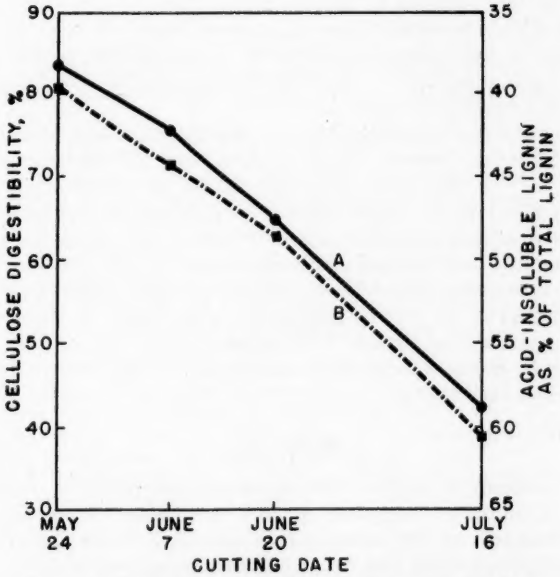

Figure 3. Changes in the in vitro cellulose digestibility (A) of timothy hay and its "acid-insoluble" lignin content expressed as percent of "total" lignin (B).

When acid-insoluble lignin, as percent of total lignin, is plotted against stage of maturity (cf. figure 3) the resulting line follows that for cellulose digestibility very closely. From this it is concluded that acid-insoluble lignin is more closely related to the decreasing digestibility of cellulose than is total lignin and that this correlation becomes more clearly evident when acid-insoluble lignin is expressed as a percentage of total lignin rather than of dry matter.

The use of N-corrected values for total and acid-insoluble lignin has little advantage as the observed changes in these values followed patterns very similar to those obtained with the ash-free values (cf. figures 1 and 2).

Returning to the differences in the digestibility of the cellulose in the brome grass and in the orchard grass hays, it is of interest that acid-insoluble lignin accounted for 60.3% and 61.4% of the total lignin in the former hays, while the corresponding figures for the orchard grass hays were 55.23% and 57.1%. Here again, the higher proportion of acid-insoluble lignin is associated with lower cellulose digestibility.

Of further interest is the data presented in table 4 concerning "non-lignin" methoxyl content of these forages. It will be seen that the level of "non-lignin" methoxyl in the two brome grass hays is almost double the level in the two orchard grass hays. It is difficult to speculate on the significance of this finding in relation to the digestibility of the cellulose in these hays, especially as the corresponding data for the timothy hay series show a trend in the opposite direction.

While the data obtained with the timothy hays indicated a fairly close relationship between lignin content and cellulose digestibility, especially in the case of acid-insoluble lignin, the differences in lignin content between first *vs.* second stage orchard grass or first *vs.* second stage brome grass appear too small to account for the changes in cellulose digestibility observed. The increased synthesis of non-lignin, methoxyl-containing components in mature forage plants which affect digestibility is a possibility. Another aspect of the over-all problem is the possibility of changes in the physical and chemical nature of the cellulose itself, which is the subject of a paper by Baker *et al.* (1958), or in the association between lignin and cellulose in the cell wall.

Summary

In an attempt to explain the decreased digestibility of cellulose in forages of advancing maturity, a study was made of the lignin and methoxyl content of two brome grass hays, two orchard grass hays and four timothy hays which were cut at different stages of maturity.

The lower digestibility *in vitro* of the second stage orchard and brome grass hays relative to that of the less mature first stage could not be explained in terms of proximate composition or lignin content. In contrast, decreasing cellulose digestibility in the case of the four timothy hays, did appear to be related to lignin content and especially to acid-insoluble lignin expressed as a percentage of total lignin.

When the acid-insoluble lignin of the brome and orchard grass hays was expressed as a percentage of total lignin, the percentage acid-insoluble lignin was higher in the brome grass hays than in the orchard grass hays showing an association between higher acid-insoluble lignin values and decreased cellulose digestibility. It was also observed that the level of "non-lignin" methoxyl in the brome grass hays was almost double that in the orchard grass hays.

It is concluded that in the mature brome and orchard grass hays studied, the differences in the lignin content of hays cut at different stages are too small to account for the observed differences in the digestibility of the cellulose.

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THE STORAGE POTENTIAL AND CITRIC ACID CONTENT OF FRACTIONATED EJACULATES OF THE BOAR [1, 2]

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DURING the past few years there has been increasing interest in artificial insemination of swine. Russian investigators (Milovanov, 1932; Nesmejanova, 1937, 1940; Rodin and Lipatov, 1935) did the original work which included investigations into storage of boar semen and the biochemistry of semen as well as studies on artificial insemination techniques and physiology of reproduction. McKenzie *et al.* (1938) investigated the reproductive physiology of the boar and the contributions made by the accessory glands. Similar investigations have been made recently by Glover and Mann (1954). General information concerning boar semen can be obtained from the monographs of Anderson (1945) and Mann (1954b), and also from recent review articles by Polge (1956a, b). A number of publications have appeared dealing with storage of boar semen (Aamadal and Hogset, 1957; Ito *et al.*, 1951; Polge, 1956a; Roy, 1955; Young *et al.*, 1957). It was recognized that satisfactory media for the storage of boar semen would differ from those used for storage of bull semen, and that the large volume of semen presented a special problem. It was also observed that the spermatozoa in the epididymis (Lasley and Bogart, 1944), and in the sperm rich fractions were more resistent to adverse conditions than were spermatozoa in whole semen (Polge, 1956a).

The following study was undertaken in order to examine certain physiological and biochemical characteristics of fractionated ejaculates.

Experimental Procedure

The experiments reported in this paper were conducted from September, 1957, to May, 1958. The study was made with four boars that were collected every Monday, Wednesday, and Friday; and four other boars with an indefinite but equally heavy collection frequency. The possible effect of the collection frequencies on storage of boar semen was not investigated. Semen used in this experiment was collected by means of an artificial vagina. Separation of the ejaculate into sperm rich and sperm poor fractions was accomplished by observing the nature of the secretions and

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[3] The authors wish to acknowledge the assistance of Dr. W. J. Tyler in the statistical aspects of this study.

[4] Departments of Animal Husbandry and Dairy Husbandry.

by changing the collection vessels during the ejaculation. All of the storage data represent semen from the sperm rich fraction only. For the purpose of presentation the experimental procedure will be subdivided into separate phases.

Phase 1. The volumes of the sperm rich and sperm poor fractions were recorded. Sperm concentrations were determined by hemocytometric counts. Both chambers of the hemocytometer were counted and the values were averaged if the difference between the two counts was 10% or less; when the differences were larger, a new count was made. Subsequently the total number of spermatozoa in each fraction and the percentage of the total spermatozoa in each were calculated.

Phase 2. Preliminary work with sodium citrate, egg yolk diluent was not successful. Several investigators have observed the beneficial effects of glycine on the livability of spermatozoa (Tyler and Tanabe, 1952; Roy and Bishop, 1954; Roy, 1955). Subsequently four diluents containing glycine were formulated; their composition is given in table 1.

TABLE 1. PERCENTAGE COMPOSITION OF THE GLYCINE-CONTAINING DILUENTS

Ingredient	Diluent			
	A	B	C	D
4% glycine solution	25	15	50	25
Heated skim milk[a]	63.75	72.25	42.5	52.5
Egg yolk	11.25	12.75	7.50	22.5

[a] Skim milk was heated to 92° C. and then cooled.

A fifth diluent, diluent E, had the following composition: Egg yolk 30%, distilled water 70%, glucose 3% by weight, sodium bicarbonate 0.15% by weight. In order to insure optical clarity all diluents were centrifuged at 2500 rpm. for 30 minutes.

Within 30–45 minutes after collection the semen was brought to the laboratory and extended 1:5 (1 ml. of semen and 4 ml. of diluent) with the respective diluents. Streptomycin sulfate was used at the rate of 1000 mcg. per ml. of diluted semen. Diluted semen was pipetted into vials in 5 ml. quantities, corked and refrigerated at 6–7° C.

The initial estimate of the percent of motile spermatozoa was made immediately after dilution (day one) and then at approximately 24-hour intervals for the next 5 days. Prewarmed slides and a stage incubator were used for all examinations. Forty-eight collections from eight boars were examined during this phase.

Phase 3. The effects of centrifugation on the sperm rich fraction and the removal of the accessory fluid on storage were investigated by using 22 collections. One ml. samples of semen were centrifuged at 1500 rpm. for 5 minutes and the supernatant was removed by suction. Respective

TABLE 2. AVERAGE VOLUMES, CONCENTRATIONS, TOTAL NUMBERS AND THE PERCENTAGES OF SPERMATOZOA IN SPERM RICH AND SPERM POOR FRACTIONS

Fraction	Volume, ml.[a]			Sperm per ml.[b] X 10^6	Total number of sperm X 10^6	Percent of total sperm
	With gel	Without gel	Percent gel			
Sperm rich	70.9	62.4	12.0	593.5	40937	79.98
Sperm poor	184.0	140.9	23.4	74.8	10245	20.02

[a] 52 collections are represented in the volume estimates.
[b] 41 samples are represented in the concentration and total number estimates.

diluents were added to make up 5 ml. in volume and the spermatozoa were resuspended by gentle mixing. Storage and evaluations were performed in the same manner and at the same intervals as in Phase 2.

Phase 4. The biochemistry of fractionated ejaculates was investigated with respect to citric acid. Citric acid was determined by the method of Saffran and Denstedt (1948). Two separate trials were performed. In Trial I semen was collected as separate 10 ml. fractions. In Trial II it was collected at half-minute intervals. The gelatinous material was separated during the collection by directing the semen from the artificial vagina onto a cheese cloth covered funnel. Fractions were classified as sperm rich or sperm poor on the basis of microscopic examination.

For the purpose of statistical analyses the percent values from the storage trials were transformed to angles and analyzed by the method of unweighted means (Snedecor, 1946). Treatment means were compared by using Duncan's New Multiple Range Test (Duncan, 1955).

Results and Discussion

Phase 1. In table 2 are given the results obtained in Phase 1.

These results fall within the ranges reported by other authors (Anderson, 1945; McKenzie *et al.*, 1938; Mann, 1954; Polge, 1956b) and point to the very high rate of spermatogenesis for the testicles of the boar. If the sperm

TABLE 3. THE PERCENT OF MOTILE BOAR SPERMATOZOA AS AFFECTED BY DILUENT AND DAY OF STORAGE [a]

Day of storage	Diluent				
	A	B	C	D	E
1	82.0	81.9	80.9	82.2	82.1
2	73.6	70.4	65.2	75.2	61.2
3	64.5	61.1	57.8	67.6	44.1
4	58.3	51.7	49.1	61.4	33.0
5	45.0	36.8	39.1	47.3	19.2
6	29.5	22.1	28.3	31.5	16.0

[a] Forty-eight collections are represented in this table.

TABLE 4. ANALYSIS OF VARIANCE OF THE PERCENT OF MOTILE SPERMATOZOA IN DIFFERENT DILUENTS [a]

Source	D.F.	Mean square				
		Day 2	Day 3	Day 4	Day 5	Day 6
Between diluents	4	259.32**	561.99**	824.97**	953.40**	371.32
Between boars	7	594.17**	1044.81**	934.89**	1125.61**	1721.83**
D x B	28	18.78	56.48	68.91	46.88	71.18
Within	200	72.38	86.15	98.6	179.79	203.74

[a] Represents data from table 3.
** Significant at the 1% level.

poor fraction were discarded, 20% of the total sperm would be lost. However, the possible gain in storage quality of the sperm rich fraction (Polge, 1956a) makes the outlook more optimistic. More careful separation and also the inclusion of more of the post sperm rich fraction could produce a further saving in spermatozoa.

Phase 2. The means of the diluent storage data are shown in table 3 while the analysis of variance and tests of the difference in the means are presented in tables 4 and 5. The data for Day 1 were not analyzed because there were no differences observed for the different diluents at the time of the first evaluation.

Examination of the data in table 3 shows that the decrease in percent of motile sperm was not uniform for the different diluents studied. No profound decrease after Day 2 as reported by Aamadal and Hogset (1957) was observed. The largest decreases occurred after Day 4 for the diluents A, B and D and after Day 1 for diluents C and E. From table 5 it is apparent that the diluents A, B and D were not significantly different from one another in their ability to support motility, and that diluents E and C were inferior in this regard. The rather pronounced deterioration

TABLE 5. COMPARISON OF TREATMENT MEANS OF BOAR SEMEN SAMPLES EVALUATED ON DIFFERENT DAYS [a, b]

Day	Diluents				
2	E	C	B	A	D
	———	———			
		———	———		
3	E	C	B	A	D
			———	———	
4	E	C	B	A	D
		———	———		
5	E	C	B	A	D
		———	———	———	

[a] Any means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different. 1% level of probability.
[b] Represents data from table 3.

of all samples by Day 6 undoubtedly accounts for the nonsignificance of diluent differences on that day.

The understanding of the action of the materials that make up a diluent is important for the purpose of diluent improvement. The beneficial effects of glycine on storage of spermatozoa of a number of species have been observed (Roy, 1955; Roy and Bishop, 1954; Tyler and Tanabe, 1952). The exact nature of glycine's action is not known but it could be related to (1) its buffering capacity, (2) its action as a chelating agent, and (3) less likely, its action as a source of energy. The failure of diluent C to support motility at a high level during storage is puzzling. Several factors could

TABLE 6. THE PERCENT OF MOTILE BOAR SPERMATOZOA AS AFFECTED BY DILUENTS, CENTRIFUGATION AND DAY OF STORAGE [a]

	Diluent and treatment									
	A		B		C		D		E	
Day	U[b]	C[c]	U	C	U	C	U	C	U	C
1	80.0	80.5	79.9	80.4	78.3	78.9	80.7	81.1	79.6	80.0
2	71.8	65.4	69.8	64.5	61.0	49.8	72.1	68.4	62.6	67.5
3	62.8	59.6	61.5	54.9	55.5	39.8	65.4	56.5	48.1	54.5
4	58.6	48.9	53.5	45.4	47.8	34.7	56.8	43.3	39.4	34.0
5	50.5	37.8	45.1	32.5	39.3	25.1	50.0	39.5	29.7	24.9
6	39.6	19.2	30.7	18.5	30.7	11.4	40.1	17.6	21.2	15.5

[a] Twenty-two collections are represented in this table.
[b] U=uncentrifuged.
[c] C=centrifuged.

be implicated (1) high level of glycine solution, (2) the low level of egg yolk, or (3) the lower percentage of milk. The interaction of these factors should be studied.

Two phenomena that frequently made microscopic evaluations difficult were the clumping of spermatozoa and the clumping of the medium. The spermatozoan clumping began in some samples as early as Day 2 and became progressively worse on subsequent days. While the clumped spermatozoa exhibited weak motility, the unclumped ones were not influenced. The medium clumped at a later time than the sperm, but was closely related to a pronounced decrease in the percent of motile spermatozoa. The nature of the medium clumping was not investigated, but it resembled protein denaturation following acid treatment.

Strictly from percent motility considerations, semen that was stored for 48 hours was satisfactory for insemination. In many cases semen 96 hours old could have been used. As to whether percent motility is a good criterion of fertility estimation is not known. Some authors tend to doubt it (Polge, 1956b; Stratman *et al.*, 1958), but present knowledge of

TABLE 7. ANALYSIS OF VARIANCE OF PERCENT OF MOTILE SPERMATOZOA AS AFFECTED BY THE DILUENT, DAY OF STORAGE AND CENTRIFUGATION

Source	D.F.	Mean square				
		Day 2	Day 3	Day 4	Day 5	Day 6
Between diluents	4	307.00**	331.92**	421.87**	409.56**	258.37
Between centrifuged and uncentrifuged	1	218.72	376.98*	1058.80*	1910.19**	3704.03**
D x C	4	76.63	119.06	24.59	68.13	122.12
Between boars	6	988.87**	1316.92**	1077.01**	1570.66**	1740.16**
B x C	6	30.72	119.52	224.40	298.74**[a]	93.74
Residual	48	27.22	36.43	29.46	26.05	29.69
Within	150	84.97	56.71	172.99	76.32	214.82

[a] This interaction is due to unusual drop in the percent of motile sperm for one boar.
* Significant at the 5% level.
** Significant at the 1% level.

TABLE 8. CITRIC ACID CONCENTRATION IN CONSECUTIVE 10-ml. FRACTIONS OF BOAR EJACULATES

Consecutive 10 ml. sample	Boar and date of collection								
	81x 2-21-58	81x 2-28-58	251 2-21-58	251 2-26-58	260 2-24-58	260 3-3-58	450 2-19-58	450 2-26-58	450 2-28-58
	(Mg. percent)								
1	76.5	20.1	49.5	138.1	59.7	97.4	187.6	182.4	50.6
2	27.8	46.0	49.5	67.5	45.0	112.3	19.6	114.8	115.4
3	44.9	63.2	113.0	94.8	33.5	119.4	68.0	187.5	206.1
4	69.0	20.1	158.0	58.8	64.9	129.0	128.9	295.5	338.2
5	120.3	36.8	162.0	113.4	107.9	127.7	213.4	339.8	326.7
6	170.1	79.9	176.0	129.9	124.1	145.2	83.5	385.2	317.5
7	189.3	99.4	195.5	184.0	127.7	189.7	92.3	286.9	366.3
8	187.2	76.4	149.0	210.8	146.6	145.2	200.0	409.7	211.9
9	207.5	27.6	135.0	207.7	129.8	145.2	266.0	388.1	163.1
10	209.1	12.1	122.0	210.8	131.9	159.4	290.7	342.0	123.5
11	212.8	6.9	116.5	221.1	124.1	111.6	20.6	92.6	112.6
12	201.6		184.0	214.9	97.9	165.2	248.4	63.1	322.1
13	201.6		174.0	198.5	62.3	154.2	274.2	186.4	139.0
14	175.4		137.5	210.8	71.7	142.6	91.8	229.0	196.4
15	166.8		147.5	213.4	100.0	101.9	215.5	342.0	127.5
16				207.7	62.3	45.2	219.6	379.5	115.4
17				228.9	74.9	15.5	283.5	423.3	216.5
18				219.6	73.3	3.9		262.5	131.5
19				238.1	80.1	69.7			117.2
20				139.2	89.5	67.7			102.8
21					65.4	84.5			
22					71.6	116.8			
23					50.3				
24					103.1				
25					75.4				
26					86.9				
Total per ejaculate	226.0	48.8	206.9	350.8	226.0	244.9	290.4	491.0	380.0

artificial insemination techniques for swine is too meager for a positive assertion.

Phase 3. It is of interest that centrifugation proved to be harmful after 2 days of storage (table 6 and 7). Besides the possibility of a harmful effect produced by the stress of centrifugation, the effect of removal of the accessory fluid should be considered. It is within reason to postulate that something was being removed with the plasma that was necessary for high survival over a period of several days. In view of these results, caution

TABLE 9. CITRIC ACID CONCENTRATION IN CONSECUTIVE HALF MINUTE FRACTIONS

Consecutive half minute intervals	Boar and date of collection								
	81x 3-28-58	251 2-28-58	251 3-4-58	260 3-5-58	260 3-28-58	382 3-17-58	450 3-7-58	450 3-10-58	450 3-14-58
	(Mg. percent)								
0.5	25.15	73.8	229.28	6.4	40.94	49.71	79.7	197.1	117.54
1.0	36.84[a]	156.1[a]	51.93	4.1[a]	50.29[a]	75.44[a]	344.9[a]	465.5[a]	309.94[a]
1.5	63.74[a]	158.5[a]	7.18	24.4[a]	91.23[a]	113.45[a]	268.4	344.4[a]	402.34[a]
2.0	67.25[a]	204.9[a]	9.84	65.7[a]	81.87[a]	167.84	310.7	250.9	457.89
2.5	64.91	200.0	28.18	76.7[a]	67.84	188.89	179.1	239.8	384.80
3.0	26.90	156.7	58.01[a]	33.7	50.29	166.08	367.9	245.6	378.95
3.5	15.79	175.6	58.01[a]	59.3	74.85	72.51		56.7	249.12
4.0	19.88	133.5	112.71	91.9	87.13	54.97		35.1	289.47
4.5	15.79	139.6	203.87	61.0	80.12	113.45		35.1	314.62
5.0	15.20	98.8	132.60	45.3	85.38	161.40		12.3	294.74
5.5		92.1	87.29	29.3	119.88	103.51		10.5	
6.0		146.3		27.3	95.91	29.82		39.2	
6.5				59.3		59.65		127.5	
7.0				67.4		41.52			
7.5				35.5		36.84			
8.0				43.6		105.26			
8.5				65.1		245.61			
9.0						156.73			
Total per ejaculate	55.1	270.6	121.5	123.3	155.9	288.9	526.6	437.4	806.6

[a] Represents the sperm rich fraction.

should be exercised in centrifuging fractionated boar semen as a means of concentrating it for dilution and storage purposes.

Phase 4. Tables 8 and 9 present raw data for the citric acid concentrations. The consecutive 10-ml. samples (table 8) show that citric acid is present during all phases of the ejaculation. The main source of citric acid in the boar is the seminal vesicles, the prostate containing a much smaller amount (Humphrey and Mann, 1949). The high content of citric acid even in the first 10 ml. of fluid (table 8) suggests that the origin of this initial citric acid is from the seminal vesicle rather than the prostate. A similar situation exists in the samples collected at half-minute intervals (table 9).

The data reported in this paper are both in agreement and disagreement with those reported by Glover and Mann (1954). It is obvious that citric acid was present in all fractions. The highest concentration usually occurs

both in the sperm rich and the post sperm fractions. Examination of the data reveals that the ejaculatory pattern for citric acid differs between boars and for different ejaculates from the same boar. The data in this paper are not in agreement with the conclusion of Glover and Mann (1954) that the "pre sperm" fraction has no seminal vesicular secretions. Mann (1954a) observed that the citric acid content of seminal vesicular fluids from five boars ranged from 137 to 1001 mg./100 ml. For prostatic tissue the value of 38 mg./100 gm. of fresh tissue was given (Humphrey and Mann, 1949). Undoubtedly analysis of prostatic secretions would reveal a variability for citric acid similar to that observed in seminal vesicular fluids, but it is not likely that the prostate is responsible for the values in the range of 150 mg. percent as observed in this study (table 8.).

Summary

A study of some physiological and biochemical characteristics of fractionated boar ejaculates was undertaken. In the sperm rich fraction the sperm concentration and total numbers of spermatozoa were 593.5 x 10^6 and 40,937 x 10^6, respectively. The respective values for the sperm poor fraction were 74.8 x 10^6 and 10,245 x 10^6.

Results from storage trials showed that diluents containing various proportions of glycine, skim milk and egg yolk differed significantly in their ability to maintain motility.

Centrifugation and the removal of accessory fluids were significantly detrimental after the second day of storage.

The citric acid content of successive 10-ml. samples and of fractions collected at ½-minute intervals was investigated. Citric acid was found in all samples, the secretory peak occurring in the sperm rich fraction and immediately thereafter.

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AN ATTEMPT TO INDUCE URINARY CALCULI FORMATION IN STEERS WITH THE EFFECTS ON CERTAIN BLOOD AND URINE CONSTITUENTS [1]

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URINARY calculi present a problem of considerable magnitude in the livestock industry. Sheep are especially susceptible to this condition, but cattle are less affected. Even so, in a recent survey among the members of the American Natonal Cattlemen's Association (Ensminger *et al.*, 1955), urinary calculi ranked fifth in importance among the nutritional diseases and ailments reported. The Great Plains area reported the highest incidence of calculi.

Lindley *et al.* (1953) reported a high incidence of urinary calculi in sheep supplemented potassium acid phosphate (K_2HPO_4). Phosphorus was considered responsible for the results obtained by these workers; however, later work (Elam *et al.*, 1956) indicated that both phosphorus and potassium in the potassium acid phosphate were responsible. Phosphorus supplementation resulted in fewer cases of calculi than potassium supplementation, but the greatest incidence occurred in lots of sheep fed rations supplemented with both these mineral elements.

Osborne and Mendel (1917) reported the occurrence of urinary calculi in rats that had been without an adequate source of fat-soluble vitamin. Higgins (1935, 1951) reported that a vitamin A deficiency predisposed to calculi formation in rats. Schmidt (1941) reported that a vitamin A deficiency caused urinary calculi in castrated male goats, whereas several workers (Beeson *et al.*, 1943; Lindley *et al.*, 1953) found no relation between such deficiency and urinary calculi in sheep. Swingle and Marsh (1956) concluded that in range steers, vitamin A deficiency *per se* did not predispose to the formation of urinary calculi.

The objectives of the present study were (1) to determine if rations which were predisposing to urinary calculi formation in sheep would cause the formation of calculi in steers, (2) to compare the effects of dehydrated alfalfa, wheat straw and potassium acid phosphate in steer rations upon certain blood and urine constituents, and (3) to observe the effect of a carotene-vitamin A deficiency on the incidence of urinary calculi in steers.

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Experimental Procedure

Twenty-four Hereford steers, averaging 516 lb. in weight were randomly divided into eight groups, each of three steers, and fed the pelleted experimental rations shown in table 1 for 158 days. During the first 14 days, all steers were fed a small amount of wheat straw in addition to their respective rations. The steers were group-fed twice daily, and feed consumption was recorded. Water was available from automatic waterers.

The variables included in the rations were potassium acid phosphate (K_2HPO_4), wheat straw versus dehydrated alfalfa as the roughage, and supplemental vitamin A. Potassium acid phosphate [4] was added to half the

TABLE 1. COMPOSITION OF STEER RATIONS [a]

Ingredients	Ration number							
	1 [b]	2	3 [b]	4	5	6	7	8
Wheat	18	15	18	15	18	18	18	18
Oats	17	17	17	17	17	17	17	17
Linseed meal	14	14	14	14	14	14	14	14
Beet pulp	25	25	25	25	25	25	25	25
Wheat straw	25	..	25	..	25	..	25	..
Dehydrated alfalfa	..	25	..	25	..	25	..	25
NaCl	1	1	1	1	1	1	1	1
Sodium bentonite	..	3	..	3	..	..	..	..
K_2HPO_4	..	..	2.5	2.5	2.5	2.5	..	..

[a] Values in pounds.
[b] Vitamin A concentrate added to rations 1 and 3 at rate of 700 i.u. per lb.

rations at a level (50 lb. per ton of ration) reported to cause calculi formation in sheep (Lindley *et al.*, 1953; Taysom *et al.*, 1953; Elam *et al.*, 1956, 1957). Wheat straw was included in half the rations at a level of 25% and dehydrated alfalfa was included in the other half at the same level. Half of the wheat straw rations (1 and 3) were supplemented with 700 i.u. of vitamin A per lb. of ration. Sodium bentonite was included in two of the rations containing alfalfa (2 and 4), and its effects are reported elsewhere (Erwin *et al.*, 1957).

Each ration was analyzed for its content of calcium, phosphorus, magnesium, potassium, sodium, and chloride. Calcium and phosphorus were determined according to the methods described by Fister (1950). Chloride was determined by the method listed by Fister (1950) on samples previously ashed according to the A.O.A.C. recommendations. Sodium and potassium analyses were carried out flame-photometrically on samples treated according to the method of Shoji and Nakata (1954). Magnesium was determined by the method described by Fister (1950), samples being ashed according to the method of Shoji and Nakata (1954).

The steers were observed daily for symptoms of urinary calculi. After

[4] Appreciation is expressed to Merck and Company, Rahway, N. J. who supplied the pure K_2HPO_4 used in this experiment.

55 days on experiment they were placed in metabolism stalls (Erwin *et al.*, 1956) and a 24-hour urine sample was collected under toluene. The total 24-hour volume was measured and 100 ml. removed for pH determination with a glass electrode. Also, the surface tension of a sample of the 24-hour specimen was determined with a DuNouy tensiometer. An aliquot was frozen for further analyses.

The total solids in the urine were determined by the method described by Hawk *et al.* (1949). Calcium, inorganic phosphorus, chloride and magnesium were determined on urine samples by modifications of the

TABLE 2. THE PROXIMATE AND MINERAL COMPOSITION OF THE STEER RATIONS [a]

Constituent [b]	Ration number							
	1	2	3	4	5	6	7	8
Dry matter	88.7	87.1	87.7	87.5	88.5	88.1	89.7	89.1
Crude protein	12.1	15.2	11.9	14.6	13.5	15.4	12.4	16.3
Ether extract	1.98	2.30	1.74	2.02	1.60	2.13	2.13	2.85
Crude fiber	15.7	11.7	15.5	10.4	15.0	10.9	15.9	11.4
Ash	5.65	8.49	7.66	10.52	8.39	8.39	5.24	6.71
N.F.E.	53.27	49.41	50.90	49.96	50.01	51.28	54.03	51.84
Calcium	0.38	0.74	0.25	0.65	0.43	0.78	0.28	0.60
Phosphorus	.29	.27	.67	.67	.72	.76	.30	.33
Magnesium	.....	.28	.20	.29	.20	.32	.19	.29
Potassium	.....	.93	1.74	1.99	1.95	2.13	.93	.99
Sodium	.57	.74	.57	.66	.58	.67	.56	.51
Chloride	.89	.71	.98	.84	.98	1.11	.84	1.06

[a] Values in percent.
[b] Rations 1, 3, 5, 7 contain wheat straw, Rations 2, 4, 6, 8 contain dehydrated alfalfa, Rations 3–6 contain K_2HPO_4.

methods discussed above. Sodium and potassium analyses were done by the flame photometric technique according to the Beckman Data Sheet DU-12-B, a Beckman Model DU Spectrophotometer equipped with oxy-acetylene flame attachment and photomultiplier being used.

Blood samples were taken from all the steers 103 days after the start of the experiment. The plasma levels of calcium, inorganic phosphorus, magnesium, potassium, sodium, and chloride were determined by modification of the above methods.

At the conclusion of the trial, the animals were sacrificed. The urinary tract of each animal was taken and later examined thoroughly for calculi. A liver sample was also collected from each steer and analyzed later for carotene and vitamin A (Gallup and Hoefer, 1946).

Results and Discussion

The chemical composition of the steer rations is listed in table 2.

Two steers receiving dehydrated alfalfa died of bloat, one on the 71st day and the other on 130th day of the experiment.

No characteristic symptom of urinary calculi was noticed in the steers during the 158-day experimental period. Six steers were found to have calculi in the bladder at the time of slaughter. In every case, however, the stones were few, very small and sand like, and did not appear large enough to block the flow of urine. These six cases of calculi were equally distributed among the treatments; that is, three animals fed wheat straw, three fed dehydrated alfalfa, three fed potassium acid phosphate and three fed none had stones present.

The above results indicate that feeding a carotene deficient ration (wheat straw *vs.* Alfalfa) or a ration supplemented with potassium acid phosphate does not increase the incidence of urinary calculi in steers. The results with wheat straw lend support to the report by Swingle and Marsh (1956) that there was no relation between a vitamin A deficiency and

TABLE 3. AVERAGE CAROTENE AND VITAMIN A CONTENT OF LIVERS[a]

Ration treatment	Carotene	Vitamin A
Wheat straw	0.95[b]	0.33[b]
Dehydrated alfalfa	7.04[c]	28.00[c]
No K_2HPO_4	4.07[b]	12.11[b]
K_2HPO_4	3.42[c]	13.86[c]

[a] Values in micrograms per gram fresh liver.
[b] Average of 12 steers.
[c] Average of 10 steers.

urinary calculi in range beef cattle. Several workers (Lindley *et al.*, 1953; Beeson *et al.*, 1943) have reported no relation between urinary calculi formation and the vitamin A status of sheep.

The carotene and vitamin A content of the liver samples taken at slaughter are shown in table 3.

The vitamin A content of the liver of steers fed wheat straw was markedly less than that of those fed dehydrated alfalfa (table 3.) The average liver vitamin A of the steers fed wheat straw supplemented with vitamin A was almost as low as that of the steers not receiving the supplement. The averages for the two groups were 0.60 and 0.07 mcg. per gram of fresh liver, respectively. Thus, it appears that in spite of vitamin A supplementation these animals were vitamin A-deficient. The reason for this is not known. The deficiency of vitamin A was apparent not only from the liver analyses, but also from the appearance and action of the animals. Several of the animals fed wheat straw showed night blindness. One was completely blind. The haircoats were very rough by the end of the experiment. As a consequence, no conclusion can be drawn about the effect of the vitamin A supplement on calculi formation.

The fact that feeding potassium acid phosphate did not increase the incidence of urinary calculi is contrary to results found in sheep (Lindley *et al.*, 1953; Taysom *et al.*, 1953; Elam *et al.*, 1956, 1957). On the other hand, Black *et al.* (1947) reported results with steers which indicated that

TABLE 4. MINERAL CONTENT OF BLOOD PLASMA OF STEERS[a]

Ration treatment	Wheat straw	K_2HPO_4 + Wheat straw	Dehydrated alfalfa	K_2HPO_4 + Dehydrated alfalfa
No. of animals	6	6	6	5
Calcium	9.67	9.76	9.98	9.13
Inorganic phosphorus	8.79	9.86	8.54	10.36
Magnesium	2.50	2.43	2.21	2.14
Potassium	17.03	16.42	17.19	19.18
Sodium	310	324	319	329
Chloride	362	369	386	375

supplementing rations with phosphorus, in the form of bonemeal, controlled to a considerable extent the formation of urinary calculi. Later work (Jones *et al.*, 1953) indicated that for this purpose bonemeal was helpful when added to cottonseed hulls, but detrimental when added to silage.

One might question whether the length of the experimental period in the present study was sufficient to test for urinary calculi formation in cattle; however, in trials with sheep Taysom *et al.* (1953), Elam *et al.* (1956, 1957) reported calculi formation in 25, 32 and 33 days, respectively.

Plasma mineral levels as influenced by the treatments are listed in table 4 and table 5 shows the results of statistical analyses of these data.

The plasma levels of calcium, magnesium and chloride were not significantly altered by the different treatments. The phosphorus level was significantly ($P<.01$) increased in the plasma of steers fed potassium acid phosphate. Lindley *et al.* (1953) reported a similar effect in sheep fed potassium acid phasphate. The plasma sodium level was also significantly ($P<.05$) higher in steers receiving potassium acid phosphate in their ration. Laragh and Capeci (1955) reported that feeding potassium chloride to dogs increased the serum level of sodium, and suggested that an exchange of potassium for the sodium ion took place within the cell, producing a rise in extracellular sodium.

Plasma potassium was significantly ($P<.01$) higher in steers fed dehydrated alfalfa than in those fed wheat straw. However, it was not influenced by potassium acid phosphate. These results appear unusual and can hardly be explained from the potassium content of the rations as listed

TABLE 5. ANALYSIS OF VARIANCE OF STEER PLASMA VALUES[a]

Source	d.f.	Calcium	Phosphorus	Magnesium	Potassium	Sodium	Chloride
Total	22						
Roughage	1	0.173	0.09	0.485	1248**	81	1350
K_2HPO_4	1	.829	12.61**	.031	288	523*	28
Interaction	1	1.307	.85	.000	1027**	160	417
Error	19	.504	.93	.177	115	75	479

[a] Mean squares only.
* $P<.05$.
** $P<.01$.

in table 2. The rations containing alfalfa (even-numbered) were not markedly higher in potassium than the wheat straw rations. They were consistently higher in calcium, but whether this would account for the higher plasma potassium level of the alfalfa-fed steers is not known.

The effects of the treatments on urine values are listed in tables 6 and 7.

The influence of the various treatments on 24-hour urine volume was not statistically significant. However, the increase in urine volume that resulted when potassium acid phosphate was fed with wheat straw should be noted

TABLE 6. AVERAGE STEER URINE VALUES AS AFFECTED BY RATION TREATMENT

	Treatments			
Items	Wheat straw	K_2HPO_4 + Wheat straw	Dehydrated alfalfa	K_2HPO_4 + Dehydrated alfalfa
No. of animals	6	6	6	6
Volume, ml./24 hr.	5543	9728	9530	9125
pH	7.83	7.68	8.18	7.99
Surface tension, dynes/cm.	69.5	71.8	75.4	70.9
Solid, gm. %	5.65	4.10	3.35	4.59
Calcium mg. %	4.44	4.07	2.37	2.90
Phosphorus, mg. %	30.96	127.60	11.10	92.90
Magnesium, mg. %	1.76	0.83	1.97	0.26
Potassium, mg. %	518	649	422	749
Sodium, mg. %	522	377	377	374
Chloride, mg. %	710	497	516	521

(table 6). In fact, several interactions found in the concentrations of urine constituents appear to be a result of this effect.

The surface tension of the steer urine was not affected significantly by the type of roughage fed or by the supplement of potassium acid phosphate.

The concentration of solid material in the urine was significantly greater ($P<.05$) in the steers fed wheat straw. Moreover, there was a significant interacting ($P<.01$) between the type of roughage and potassium acid phosphate. That is, the addition of this salt to the wheat straw rations decreased the concentration of urine solids, while its addition to alfalfa increased the concentration of urine solid. These effects can be explained by the difference in urine volume.

The concentration of calcium in the urine was significantly greater ($P<.05$) from steers fed wheat straw than from those fed alfalfa (tables 7 and 8). This finding was not expected inasmuch as the alfalfa rations contained, on an average, more than twice as much calcium as the wheat straw rations (table 2). Total daily excretion of calcium was the same for the two groups. Potassium acid phosphate did not affect the concentration of calcium in the urine.

TABLE 7. ANALYSIS OF VARIANCE OF STEER URINE VALUES MEAN SQUARES ONLY

Source	d.f.	Volume	pH	Surface tension	Solids	Calcium	Phosphorus	Magnesium	Potassium	Sodium	Chloride
Total	23										
Roughage	1	171	667**	37.5	5.051*	15.74*	466	201	7	3256	4361
K_2HPO_4	1	214	167	7.0	.113	.04	4782**	10386**	31491**	3286	6479*
Interaction	1	316	4	70.1	11.886**	1.21	33	1104	5765*	2997	7140*
Error	20	1602	47	19.3	1.104	2.98	137	487	1257	2337	1326

* $P<.05$.
** $P<.01$.

As expected, the concentration of phosphorus in the urine was significantly increased ($P<.01$) when steers were fed potassium acid phosphate (table 7). The concentration of magnesium was significantly decreased ($P<.01$). The possibility exists that in the presence of the phosphate salt magnesium was absorbed less efficiently from the gastro-intestinal tract.

The potassium concentration in the urine was significantly increased ($P<.01$) in steers fed potassium acid phosphate. Also, there was a significant interaction ($P<.05$) between the type of roughage and potassium acid phosphate supplementation. The increase in urinary concentration of potassium was much greater in steers fed alfalfa than in those fed straw. This effect appears to be a result of the large increase in urine volume caused by feeding the phosphate salt with wheat straw.

Sodium concentration in the urine was not influenced significantly either by the type of roughage fed or by feeding potassium.

Chloride concentration in the urine was depressed significantly ($P<.05$) in steers fed potassium acid phosphate. Again, a significant interaction ($P<.05$) occurred between the type of roughage and potassium acid phosphate supplementation. The decrease in urinary chloride concentration was very marked only in the steers fed wheat straw. This effect also appears to be an indirect result of the influence on urine volume.

It is realized that the reported effects upon urine constituents are limited somewhat by the relatively short collection period (24 hours). Several days are usually required after steers are placed in metabolism stalls before uniform performance is attained. Perhaps total excretion would have been more significant than the percentage composition reported, but urine concentration appears to be the more important to urinary calculi formation.

Summary

Twenty-four yearling Hereford steers were randomly divided into eight groups of three and fed pelleted experimental rations for 158 days. The variables among the rations were potassium acid phosphate, wheat straw, and dehydrated alfalfa. None of the steers displayed symptoms of urinary calculi during the experiment. At slaughter, six steer had several small stones present in the bladder; but these animals were equally distributed between the treatments. Results from the analyses of blood and urine samples taken during the experiment indicated that:

1. The plasma levels of phosphorus ($P<.01$) and sodium ($P<.05$) were significantly higher in steers fed potassium acid phosphate than in those fed none.

2. The plasma level of potassium was significantly higher ($P<.01$) in steers fed dehydrated alfalfa than in those fed wheat straw.

3. The urine from steers fed alfalfa had a smaller concentration of total solids and of calcium, and was significantly more alkaline ($P<.01$) than urine from steers fed wheat straw. The differences in concentration can be explained on the basis of greater urine volume from steers fed afalfa.

4. The urine from steers fed potassium acid phosphate contained significantly increased concentrations of phosphorus and potassium, and decreased concentration of magnesium and chloride.

5. Interaction effects between type of forage and potassium acid phosphate were found in the case of total solids, potassium and chloride concentrations in the urine. All of these effects can be explained by changes in urine volume.

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THE EFFECT OF DELAYED SHEARING OF EWES AND DAYTIME COOLING OF RAMS ON LATE SPRING BREEDING PERFORMANCE

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SHEEP are seasonally polyestrous, preferring to breed in the fall. Breeds vary in the length of their active breeding season. In recent years, sheepmen have attempted to time the breeding season to produce lambs in the fall or winter. Where a limited breeding season in the late spring or summer has been used, a poor lamb crop has often resulted. Whether the ewes, the rams, or both should be incriminated for this poor reproductive behavior is not known. The detrimental effect of heat on reproduction has been demonstrated. Dutt and Simpson (1954) and Dutt and Bush (1955) demonstrated that rams subjected to reduced environmental temperatures (45°–48° F.) produced a higher fertilization rate and lower embryonic mortality than noncooled rams during mid and late summer. Dutt *et al.* (1956) working with ewes and Hulet *et al.* (1956) working with rams have demonstrated improved reproductive performance by shearing the animals shortly before breeding in late summer. The purpose of this study was to determine if a slight daytime cooling of rams under field conditions and delayed shearing of ewes would improve the reproductive performance of sheep bred during late spring and early summer.

Materials and Methods

The ewe flock used in this study is located at the Ft. Reno Experiment Station, El Reno, Oklahoma. In April and May 1955, 100 high grade Rambouillet and 100 Rambouillet X Panama-Rambouillet (hereafter called RPR) yearling ewes were purchased from separate ranches in the Del Rio area of Texas. A description of the management of the flock was reported by Harrington *et al.* (1958) except as regards the specific management of the rams and ewes relative to the breeding season.

Purebred Dorset rams (six different pairs, two pairs used as yearlings and again as two-year-olds) have been used exclusively. To study the effect of temperature on ram performance, the ewes were randomly divided into groups and a pair of rams randomly assigned to each group. Rams of a pair were twins or half brothers selected to be about equal in age, size, and apparent maturity. The temperature treatment was imposed

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on the rams from about 8:00 a. m. to 5:00 p. m. daily starting about 3 weeks before the breeding season began. The two rams of a pair were rotated (seven pairs were rotated every 4 days, one pair was rotated daily) with the assigned group of ewes. Marking harnesses were used and records made each morning of ewes mated the previous night. Marked ewes were removed when rams were rotated and all removed ewes returned to their groups on the 16th and 32nd days of the breeding season.

One randomly selected ram of each pair was kept during the day in a double-walled room in the sheep barn. The room was about 11 ft. by 18 ft. with a ceiling 8 ft. high and a concrete floor. The other ram of each pair was kept in a stall of equal dimensions in the same barn. Lighting conditions were similar. In 1955, a small evaporative cooler was used

TABLE 1. THE MEAN MAXIMUM TEMPERATURES AND THEIR STANDARD DEVIATIONS IN THE RAMS' QUARTERS DURING THE BREEDING SEASONS [a]

Dates	Cooled room		Barn stall	
	Mean max. temperature	Standard deviation	Mean max. temperature	Standard deviation
1955 (5/23 to 7/10)	78°	5.9	81°	7.5
1956 (5/22 to 7/9)	77°	6.4	88°	8.4
1957 (6/2 to 7/3)	77°	6.9	86°	9.2

[a] All rams were subjected to the same temperatures from 5 p.m. to 8 a.m. each day.

to reduce the temperature in the cooled room. In 1956 and 1957, a half-ton refrigerated air conditioner was used. Table 1 shows the mean maximum temperatures that prevailed during the three breeding seasons. The standard deviations given describe the distributions of the temperatures except that day to day variations in temperatures go in cycles rather than at random.

During the first two years, the breeding season was 48 days long. During the last year, a 32-day breeding season was used. The rams that were used each night were turned in with the ewes about 5:30 p. m. and removed the following morning at about 7:30 a. m. The rams were fed and watered during the day in their room or stall. The groups of ewes grazed during the day in small pastures where shades were available.

During the latter two years one-half of each group of ewes were subjected to a test involving the time that they were sheared. In 1956, one-half of the ewes were sheared on April 7 and the remaining half were sheared on May 12. The weather during the 1956 breeding season was hotter than normal. In 1957, the ewes were re-randomized on the basis of source and previous breeding performance and one-half sheared on April 6. The remaining half were sheared on May 22. The average temperatures during the 1957 breeding season were slightly cooler than normal.

The management of the flock was such that reliable records were obtained as to when a ewe mated (but not how many times in one night) and to which ram. A few lambs were born whose sires could not be ascertained. In such instances, the records were deleted from the data on the sires. If either ram of a pair failed to perform normally for a nontreatment cause, all data for the group were deleted.

The fertility of the rams was checked biweekly from about a month before until the end of the breeding season. Semen samples were collected by means of an electro-ejaculator and evaluated for motility and morphology.

TABLE 2. THE BREEDING PERFORMANCE OF COOLED AND NONCOOLED RAMS MATED TO TWO DIFFERENT KINDS OF EWES

	Ram treatment			
	Noncooled		Cooled	
Breeding of ewes	No. effective matings[a]	No. lambs	No. effective matings[a]	No. lambs
RPR	103	77	118	101
Rambouillet	103	83	140	122
Total	206	160	258*	223**

[a] Matings that could have resulted in conception.
* $P<.02$.
** $P<.01$.

Results and Discussion

The Effect of Cooling the Rams. Eight pairs of rams have been tested over a three-year period to evaluate the advantage of keeping rams in a cool environment during the day. The cooled and noncooled rams have had equal opportunities relative to number of ewes mated. Table 2 gives the results from this portion of the study. The cooled rams showed more agressiveness and sired more lambs. There were 21% more matings made by cooled rams and these matings resulted in 39% more lambs. Sixty-six percent of the ewes mated to cooled rams lambed while 62% of the ewes mated to noncooled rams lambed. Although the difference between 62 and 66 is not significant by ordinary tests it may be real. Six of the eight cooled rams had conception rates as high or higher than their noncooled mates. Further the two noncooled rams with higher conception rates had made 29 and 34% fewer matings than their mates. Twenty percent of the matings by cooled rams produced twins as compared to 17% for the noncooled rams. Again, the consistency of the data suggests that there may be a real difference even though the difference found was not significant. The performance of the rams is presented by groups of

ewes to show that the improved performance was obtained with both kinds of ewes. It appears that the Rambouillet ewes responded slightly more than the RPR ewes. This suggested interaction is not statistically significant.

The mechanism by which the cooling of the rams aids breeding performance is vague. Part of the improvement can be accounted for by the increased number of matings. The improved conception rate is harder to explain. Semen samples taken before and during the breeding season and evaluated for motility and morphology have not suggested any differences in quality between cooled and noncooled rams. These findings support suggestions by Hulet *et al.* (1956) and El-Skeikh and Casida (1955) that fertility may be appreciably changed with little or no change

TABLE 3. THE EFFECT OF TIME OF SHEARING ON THE REPRODUCTIVE PERFORMANCE OF TWO KINDS OF EWES

	Time of shearing					
	Early April			Mid-May		
Breeding of ewes	No. ewes	No. ewes lambing	No. lambs	No. ewes	No. ewes lambing	No. lambs
RPR ewes	96	71	83	95	85	107
Rambouillet ewes	93	77	102	99	85	119
Total	189	148	185	194	170	226*

* $P \cong 0.08$. After adjustment for differences in the total number of ewes in the two groups.

noted in semen quality. The improved fertility as shown in this study could be due to an increase in fertilization rate or a lowered embryonic mortality in the ewes bred to cooled rams. Further work is under way to determine this answer.

The Effect of Delayed Shearing of the Ewes. As previously indicated, during the 1956 and 1957 breeding seasons one-half of the ewes were shorn in early April and the others shorn 10 days before the initiation of the breeding season. Table 3 shows the results obtained with the two kinds of ewes. In 2 years, 189 early-sheared ewes produced 185 lambs whereas 194 late-sheared ewes produced 226 lambs. After adjusting the data for different numbers of total ewes, the Chi-Square test indicates that a deviation of this magnitude or larger would occur about one time out of twelve if there were truly no differences due to time of shearing. The increased lamb crop resulting from delayed shearing of the ewes came as a result of improved performance in two ways. More of the late shorn ewes lambed and there were more twins born to these ewes. In one year's data there were significantly more twins born to late shorn

ewes. The early and late shorn ewes exhibited essentially the same willingness to mate.

The cause or causes of the improved performance resulting from delayed shearing are not known. Conceivably, there could be an increased ovulation or fertilization rate or a lowered embryonic mortality. The late shorn ewes gained more weight during the breeding season than the early shorn ewes. Even though the difference was only about 2 lb., this may indicate that the late shorn ewes were more successfully flushed. Further studies are under way to elucidate the cause or causes of the improved performance.

TABLE 4. A 2 X 2 X 2 PRESENTATION OF THE RESULTS OF FIRST SERVICE ONLY AS A MEASURE OF REPRODUCTIVE PERFORMANCE AS EFFECTED BY KIND OF EWES, TIME EWES WERE SHEARED AND RAM COOLING

	RPR ewes sheared:		Ramb. ewes sheared:	
Ram treatment	Early	Late	Early	Late
Noncooled				
Matings	31	30	22	26
Lambed to 1st service	20	19	15	20
No. sets of twins	2	5	1	7
Cooled				
Matings	35	37	36	43
Lambed to 1st service	23	27	22	29
No. sets of twins	2	10	10	14

Ewes Lambing to First Service. These data were also analyzed on the basis of first service (within a breeding season) only. This measure of reproductive efficiency may contain less bias than when all matings are used if one is interested in the interactions between major variables. Analysis of all first service records for the years 1956 and 1957 indicated essentially the same conclusions as those already discussed and presented in tables 2 and 3.

Table 4 presents these results in a 2 x 2 x 2 arrangement to show that there were no significant interactions present. If interactions are present, they cannot be detected with any reasonable degree of certainty in these data. The only 2 x 2 interaction suggested is between the effects of ram cooling and kind of ewes. There was an increase in the proportion of Rambouillet ewes that were mated by cooled rams as compared to the RPR ewes.

The results presented here plus observations not heretofore mentioned indicate that the sheepman who is trying to breed in May and June should pay more attention to management than is usually considered

necessary during fall breeding. The ewes in this flock have shown a willingness to mate that resembles that shown during the fall breeding season. The proportion of ewes lambing to first service (or any other service) is low but quite variable among ram groups. Either there is a low conception rate or a high embryonic mortality. The latter is suspected but there are indications that the conception rate is low also.

The rams have varied considerably in willingness to mate and ability to settle ewes to first service. If the two rams of a pair mated with similar numbers of ewes, the cooled ram has always settled a higher proportion to first service. Two noncooled rams have shown high lambed-to-first-service records but in both cases they made very few first matings. Any ram in the 3 years of data that has made excessive matings has usually had a low ability to settle ewes. The number of matings here indicated as excessive would not be considered excessive under fall breeding conditions.

It is suggested that if we are to solve the problem of getting a good lamb crop, all born in the fall, more information is needed relative to the management of both rams and ewes. There is a great need for research to determine the effect of many management practices on the ovulation and fertilization rates and on embryonic mortality.

Summary

The study extended from 1955 to 1957 and involved the breeding and lambing records from eight pairs of Dorset rams mated to 45-50 ewes per pair. The breeding season varied in length from 32 to 48 days but was always in the time interval between May 20 and July 12. One-half of the ewes were high grade Rambouillets and the other one-half were Rambouillet X Panama-Rambouillets (RPR). Rams were yearlings except in 1957 when two-year old rams were used.

One ram of each pair was kept in a cooled room from 8:00 a. m. to 5:00 p. m. daily where the temperature seldom got over 85° F. The other ram of each pair was kept in a stall of equal size and similar light. Cooled rams produced more lambs primarily because they made more matings. They also produced more lambs per mating (nonsignificant).

One-half of the ewes were sheared in early April and the other one-half were sheared about 10 days before breeding began. The late shorn ewes produced more lambs ($P \cong 0.08$) because of a higher frequency of twins and also more of them lambed per mating (nonsignificant).

A study of first service records as a measure of reproductive performance, substantiated the foregoing results and also indicated that interactions are probably slight or non-existent between the effects produced by cooling the rams, late shearing the ewes and the kinds of ewes that were used.

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THE EFFECT OF ESTROGENIC AND ANDROGENIC COMPOUNDS UPON GROWTH AND CARCASS COMPOSITION OF SWINE [1, 2]

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THE increasing demand of the consumer for leaner cuts of pork has created a wide interest in the production of swine with a more favorable ratio of lean to fat. In view of the anabolic response of ruminants to stilbestrol and of rats to testosterone and certain of its derivatives, three feeding trials were conducted to determine whether certain testosterone analogs, combinations of stilbestrol and testosterone and late castration of boars would improve the growth rate, feed efficiency and carcass quality of growing-finishing swine.

Experimental

Trial I. Sixty weanling linebred Hampshire pigs averaging approximately 40 lb. liveweight at 9 weeks of age were divided equally according to sex and weight into six lots each of 10 pigs. A 16% protein basal ration consisting of 78.25% ground shelled corn, 13.0% soybean meal, 6.0% meat and bone scraps, 2.0% dehydrated alfalfa meal, 0.5% iodized salt plus added B-vitamins, vitamin B-12, antibiotics and zinc oxide was fed to all groups up to an average liveweight of 125 lb. From 125 lb. average liveweight to the final market weight of 210 lb., the protein in the ration was reduced to 14%. Lot treatments in mg. per lb. of ration were as follows:

Lot I, basal ration; Lot II, basal plus 5.0 mg. methyl testosterone; Lot III, basal plus 0.6 mg. 9 alpha fluoro-11 beta-hydroxy-17 alpha-methyl testosterone; Lot IV, basal plus 0.4 mg. 17 ethyl-19 nortestosterone; Lot V, basal plus 2.0 mg. 17 ethyl-19 nortestosterone; Lot VI, basal plus 0.6 mg. 11 beta-hydroxy-17 alpha-methyl testosterone.

Final summary of the growth data was made at the time of removal of the first pig from experiment. This procedure was followed in all three trials.

At a weight of approximately 205 lb., each of the pigs was slaughtered and standard carcass measurements and weights of primal cuts were obtained. One-half of each carcass was boned out, skinned, ground and

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thoroughly mixed for sampling. Individual samples were analyzed for percent of moisture, ether extract, protein and ash, and the iodine number of the extracted fat was determined by the Hanus method outlined by the Association of Official Agricultural Chemists.

Trial II. A group of 60 purebred Duroc weanling pigs which included 10 intact male pigs was divided into six lots of 10 pigs each according to weight. Each lot averaged 36 lb. initially at approximately 10 weeks of age and was *ad libitum* fed separately, shelled corn, mineral mixture (40 parts steamed bonemeal, 40 parts limestone and 20 parts iodized salt) and a protein supplement consisting of 47.25% soybean meal, 40.0% meat and bone scraps, 10.0% dehydrated alfalfa meal, 2.0% iodized salt, plus B-vitamins, vitamin B-12, antibiotics and zinc oxide. Sex divisions and treatments for each lot were as follows:

Lot I, basal ration listed above (barrows and gilts); Lot II, basal plus 1.0 mg. stilbestrol per lb. of protein supplement (barrow); Lot III, basal plus 1.0 mg. stilbestrol per lb. of protein supplement (boars). Castrated at 172 lb. average liveweight; Lot IV, basal plus 1.0 mg. stilbestrol and 20.0 mg. methyl testosterone per lb. of protein supplement (barrows and gilts); Lot V, basal plus 1.0 mg. stilbestrol per lb. of protein supplement and implants of 50 mg. testosterone propionate per pig (barrow and gilts); Lot VI, basal plus implants of 3.0 mg. stilbestrol and 50 mg. testosterone propionate per pig (barrows and gilts).

In an attempt to markedly suppress testes size of the boars (Lot III), the initial level of stilbestrol was increased to 2 mg. per lb. of supplement on the 44th day of experiment, 3 mg. on the 53rd day and 5 mg. from the 70th to the 84th day. On the 84th day the boars were castrated, individual combined testes weights were recorded and sections of each testis were obtained for histological study; following castration the boars were fed 1.0 mg. diethylstilbestrol (DES) daily until slaughter.

As each pig reached a weight of 205 lb. to 215 lb. (210 lb. average), a live probe for backfat depth was taken at three points (shoulder, last rib and last lumbar regions) with the Lean Meter described by Andrews and Whaley (1954).

Trial III. Sixty weanling purebred Duroc pigs averaging 46 lb. at 9 weeks of age were equally divided according to liveweight and sex into six lots each of 10 pigs. A basal ration identical in composition to the ration fed in Trial I was provided *ad libitum* for all groups. In addition to the basal ration, Lot I to VI received, respectively, 0, 0.3, 0.6, 0.9, 1.2 and 1.8 mg. of 11 beta-hydroxy-17 alpha-methyl testosterone per lb. of ration. When a final weight of 205 to 215 lb. (210 lb. average) was attained by each pig, measurement was made for live backfat depth as described for Trial II, and the pigs from Lot I (control) and Lot IV (0.9 mg. level) were slaughtered and processed according to the procedure outlined for Trial I. However, a determination was not made of the iodine number of the extracted fat.

Results

Trial I. The data are summarized in table 1. Although the differences in rate of gain between treatments were not significant, it should be pointed out that the animals in Lot V, which received the highest level of 17 ethyl-19 nortestosterone, grew less rapidly and consumed more feed per 100 lb. live weight gain than those in any other group.

TABLE 1. EFFECT OF TESTOSTERONE DERIVATIVES ON GAIN, FEED EFFICIENCY AND CARCASS QUALITY.[a] TRIAL I

Items	Treatment in milligrams per pound of ration					
	Lot I Control	Lot II 5.0 mg. methyl testosterone	Lot III 0.6 mg. 9d-fluoro-11B-hydroxy-17 methyl testosterone	Lot IV 0.4 mg. 17 ethyl-19 nor-testosterone	Lot V 2.0 mg. 17 ethyl-19-nor-testosterone	Lot VI 0.6 mg. 11B-hydroxy-17 testosterone
Initial wt., lb.	39.6	39.9	39.8	39.6	39.6	39.6
Final wt., lb.	192.8	189.8	189.4	191.6	179.2	193.3
Total gain, lb.	153.2	149.9	149.6	152.0	139.6	153.7
Av. daily gain, lb.	1.70	1.67	1.66	1.69	1.55	1.71
Av. daily feed, lb.	6.19	5.96	6.02	5.82	5.86	6.18
Feed per 100 lb. gain, lb.	363.3	357.8	362.1	344.4	378.0	361.8
Mg. hormone per day	0	29.0	3.6	2.3	11.7	3.7
Slaughter data						
Pre-kill wt., lb.	205.4	204.1	203.8	205.3	206.8	206.0
Chilled wt., lb.	148.1	142.9	144.0	145.3	145.6	148.6
Dressing percent	72.1	70.2	70.8	71.0	70.4	72.1
Carcass measurements						
Carcass length, in.	29.4	29.9	29.5	29.6	29.4	29.5
Hind leg length, in.	20.0	20.1	20.0	19.9	19.8	20.0
Av. backfat depth, mm.	39.3	36.5	37.5	39.1	40.1	39.1
First rib, mm.	47.6	45.3	47.5	47.7	49.9	48.6
Last rib, mm.	36.3	32.9	33.4	35.3	36.9	35.3
Last lumbar, mm.	34.0	31.2	31.6	34.4	33.4	33.4
Lean cuts (4 above)	51.3	52.9	52.0	51.7	51.9	51.1
Belly	16.0	16.0	15.7	16.3	15.4	16.6
Fatback	9.4	9.0	9.1	9.7	10.0	9.0
Jowl	3.0	3.1	3.2	3.1	3.1	3.2
Chemical analysis edible portion						
Moisture, %	38.0	39.7	39.0	39.1	38.2	38.6
Fat, %	46.7	44.9	46.2	46.5	46.8	46.2
Protein, %	13.1	13.8	13.8	13.0	13.6	13.7
Ash, %	0.56	0.59	0.56	0.58	0.56	0.58
Iodine no. of fat[b]	57.8	61.7	59.8	57.8	59.9	57.2

[a] Ten pigs per lot per 90 days.
[b] L.S.D.—5% for iodine no. is 2.3; at 1%, 3.0.

None of the differences between lot averages for dressing percentage, various carcass measurements, percent of primal cuts and percent of moisture, fat, protein and ash were significant. Nevertheless, comparison of data for backfat depth, percent of ham, loin, four lean cuts (ham, loin, Boston butt and picnic) and fat back as well as percentages of moisture, protein and fat in the edible portion, revealed that the group fed

5.0 mg. methyl testosterone per lb. of ration (Lot II) showed a tendency toward greater leanness when compared with the controls (Lot I).

Several differences between sexes were noted. Barrows grew significantly faster than gilts ($P<0.05$) and had a significantly lower percent of ham ($P<0.01$), lower percent of loin ($P<0.05$), lower percent of four lean cuts ($P<0.01$), higher percent of belly ($P<0.05$), higher percent of fatback ($P<0.01$) and lower percent of protein ($P<0.05$) than gilts. Statistical analysis of variance revealed a significant interaction of sex and treatments for average backfat depth ($P<0.05$), backfat depth at the last rib region ($P<0.05$), backfat depth at the last lumbar ($P<0.01$) and percent fatback ($P<0.05$).

During processing, several of the carcasses from the lot fed methyl testosterone (Lot II) were observed to be softer than those of other treatments. Iodine numbers of the extracted fat of all carcasses were determined to find if this increase in carcass softness was due to an increased deposition of unsaturated fats. Results of this analysis indicated that the group fed methyl testosterone (Lot II) had fat depots with a significantly higher ($P<0.01$) iodine number than the untreated controls (Lot I). Differences between the controls and pigs receiving other testosterone derivatives were not significant.

There were no recognizable external side effects due to any of the treatments.

Trial II. Data for this trial were summarized at the time of castration of the boar lot (84th day) as well as at the time of removal of the first pig at 205 to 215 lb. (94th day). Two types of analysis of variance were used for this trial. First, a single classification analysis of variances was used in which sex was not considered a source of variation. Another method was applied in which the lots containing barrows and boars (Lots II and III) were analyzed separately with a single classification, analysis of variance and the remaining four lots by the proportionate subclass analysis of variance with sex being considered a source of variation.

Prior to castration on the 84th day of the experiment the DES-fed boars in Lot III had gained 0.10 lb. daily more than the DES-fed barrows. Castration at an average weight of 172 lb. proved to be a severe stress and rate of gain was markedly reduced in the post-operative period and thus affected rate of gain as shown in table 2. Although data were not included, approximately the same difference in gain was maintained between these two lots up to the final average weight of 210 lb.

Barrows fed stilbestrol (Lot II) consumed more corn per day than boars fed stilbestrol (Lot III) and somewhat more than all other groups. The pigs fed stilbestrol and testosterone (Lot IV) consumed approximately 11% less corn than the controls (Lot I). Protein supplement consumption was nearly the same for all groups.

Stilbestrol-fed boars (Lot III) required 7% less total feed at the time of castration but nearly the same amount of total feed per 100 lb. gain

as the stilbestrol-fed barrows (Lot II) at the time of final summary. The pigs fed stilbestrol and testosterone (Lot IV), those fed stilbestrol and implanted with testosterone (Lot V) and those implanted with both hormones (Lot VI) required approximately 4 to 5% less total feed per 100 lb. gain than controls.

Single classification statistical analysis of variance of all six lots revealed no significant differences in the average backfat depth or backfat

TABLE 2. EFFECT OF VARIOUS COMBINATIONS OF STILBESTROL AND TESTOSTERONE ON GAIN, FEED EFFICIENCY AND LIVE BACKFAT DEPTH.[a] TRIAL II

Items	Lot I Barrows and gilts. Control	Lot II Barrows fed stilbestrol	Lot III Boars fed stilbestrol	Lot IV Barrows and gilts fed stilbestrol and testosterone	Lot V Barrows and gilts fed stilbestrol. Implanted with testosterone	Lot VI Barrows and gilts implanted with stilbestrol and testosterone
Initial wt., lb.	36.3	36.1	36.7	35.8	36.2	36.3
Final wt., lb. (94 days)	175.4	183.2	174.7	170.9	182.3	180.0
94-day av. daily gain, lb.	1.48	1.57	1.47	1.44	1.55	1.53
84-day av. daily gain, lb.	1.44	1.51	1.61	1.38	1.50	1.49
Average daily feed consumption						
94-days, corn, lb.	3.88	4.00	3.62	3.46	3.87	3.82
94-days, supplement, lb.	0.85	0.88	0.92	0.88	0.91	0.85
84-days, corn, lb.	3.70	3.73	3.59	3.29	3.73	3.63
84-days, supplement, lb.	0.81	0.84	0.94	0.88	0.86	0.84
Feed per 100 lb. gain						
94-days, total, lb.	319.7	312.3	309.9	302.3	307.6	306.0
84-days, total, lb.	312.8	302.3	281.7	301.5	305.7	301.0
Live backfat probe						
Av. backfat depth, in.	1.52	1.60	1.42	1.45	1.54	1.49
Shoulder, in.	1.82	1.87	1.77	1.76	1.80	1.77
Last rib, in.	1.43	1.53	1.29	1.34	1.47	1.40
Last lumbar, in.	1.30	1.42	1.19	1.24	1.36	1.30

[a] Ten pigs per lot for 94 days.

depth at any of the points of measurement. However, when stilbestrol-fed barrows (Lot II) were compared separately with stilbestrol-fed boars (Lot III), the boars had significantly less average backfat depth ($P<0.05$), less backfat depth at the last rib region ($P<0.05$) and less backfat depth at the last lumbar region ($P<0.05$). No significant differences in backfat depth were found when the data for the other four lots were analyzed separately.

All DES treated pigs showed mammary stimulation and in addition enlargement of the vulva in the females. The combinations of DES and testosterone propionate (TP) and DES and methyl testosterone produced the maximum stimulation of the vulva, and the latter treatment was characterized by an upturning of the ventral tip of the vulva. For the most part, observed side effects in each of the treated groups were not extreme

and did not fully develop until after the 2nd week of the trial. The boars did exhibit sex drives during the period just prior to castration.

Prior to slaughter the DES-fed boars and the animals which received DES and TP appeared longer in body and trimmer in the jowl and belly than those in other groups.

The absence of a group of normal boars for comparison with those fed DES and castrated at an average weight of 172 lb. limits the conclusions as to degree of testicular inhibition, if any. It is evident that the testes did not remain infantile as would have been expected had there been marked pituitary gonadotropin reduction. There was an abundance of primary and

TABLE 3. EFFECT OF 11B-HYDROXY-17 METHYL TESTOSTERONE ON GAIN, FEED EFFICIENCY AND CARCASS QUALITY.[a] TRIAL III.

	11B-hydroxy-17 methyl testosterone per pound of ration					
Items	Lot I Control	Lot II 0.3 mg.	Lot III 0.6 mg.	Lot IV 0.9 mg.	Lot V 1.2 mg.	Lot VI 1.8 mg.
Initial wt., lb.	46.2	46.2	46.2	46.4	46.5	46.2
Final wt., lb.	178.8	172.0	177.6	183.2	178.7	177.2
Total gain, lb.	132.6	125.8	131.4	136.8	132.2	131.0
Av. daily gain, lb.	1.82	1.72	1.80	1.87	1.81	1.79
Av. daily feed, lb.	6.25	5.73	6.13	6.25	6.07	5.82
Feed per 100 lb. gain, lb.	344.3	332.3	340.6	333.4	335.4	324.7
Mg. hormone per day	0	1.7	3.7	5.6	7.3	10.5
	Live backfat probe					
Av. backfat depth, in.	1.70	1.52	1.65	1.62	1.57	1.65
Shoulder, in.[b]	2.12	1.93	2.10	2.05	1.99	2.11
Last rib, in.	1.57	1.39	1.50	1.45	1.46	1.49
Last lumbar, in.	1.40	1.26	1.36	1.35	1.28	1.35
	Slaughter data					
Pre-kill wt., lb.	204.0			209.6		
Chilled carcass wt., lb.	148.9			152.6		
Dressing percent	73.0			72.8		
	Carcass measurements					
Carcass length, in.	28.6			28.8		
Hind leg length, in.	19.7			20.0		
Av. backfat depth, in.	1.81			1.73		
First rib, in.	2.33			2.24		
Last rib, in.	1.62			1.57		
Last lumbar, in.	1.47			1.37		
	Wholesale cuts as percent of carcass					
Ham	18.5			19.1		
Loin	15.2			15.3		
Boston butt	7.2			7.3		
Picnic	9.7			10.1		
Lean cuts (above)	50.6			51.8		
Belly	15.2			14.9		
Fatback	9.6			9.0		
Jowl	3.2			3.1		
	Chemical analysis edible portion					
Moisture, %	38.0			38.8		
Fat, %	50.2			48.9		
Protein, %	11.1			11.2		
Ash, %	0.54			0.56		

[a] Ten pigs per lot for 73 days.
[b] L.S.D., 5% for shoulder probe, 0.14 in.

secondary spermatocytes in all tubules, but spermatids and spermatozoa were present in only an occasional tubule. The rate of spermatogenesis may have been decreased slightly.

Trial III. None of the differences in the average daily gains between the controls and the lots fed the different levels of 11 beta-hydroxy-17 alpha-methyl testosterone were statistically significant (table 3). The lower average daily gain and daily feed consumption observed in the lot fed the 0.3 mg. level and slightly improved feed efficiency of the lot fed the 1.8 mg. level when compared to controls (Lot I) could be attributed to sampling error.

All of the treated groups had less average live backfat than the controls (table 3). However, the only significant difference was at the shoulder measurement ($P<0.05$) between the lot fed 0.3 mg. (Lot II) and the controls (Lot I). A difference approaching this level of significance was found between the control group and the group fed 1.2 mg. per lb. of ration (Lot V) when backfat was measured at the shoulder region. A comparison between the average backfat depth measured with the live probe and measurement on the chilled carcass for the control lot (Lot I) and the lot fed 0.9 mg. per lb. of ration (Lot IV) revealed that chilled carcass measurements were 0.11 inches greater for each lot than live probe measurements. The greatest difference in measurement by these two methods occurred at the shoulder (first rib).

No significant differences were determined between the control lot (Lot I) and the lot fed 0.9 mg. per lb. of ration (Lot IV) for dressing percentage, carcass measurements, percent of primal cuts or chemical analyses of edible portion of the carcass. A close survey of the data for backfat depth and percent of ham, loin, Boston butt, picnic, four lean cuts, belly, fatback, jowl, moisture and fat revealed that the group fed the hormone tended to possess greater carcass leanness.

Combining the sexes for all treatments revealed that barrows had a significantly greater live backfat depth at the last lumbar region than gilts ($P<0.05$). No external effects of hormone treatment were noted.

Discussion

In general a wide variety of androgens have been shown to increase nitrogen retention in the rat, dog and man (Hershberger *et al.*, 1953; Sala and Baldratti, 1957; Saunders and Drill, 1957).

As summarized by Saunders and Drill (1957) several androgens, especially 19-nortestosterone and 17-ethyl-19 nortestosterone have shown a relatively high anabolic response and a relatively low androgenivity in rats. However, the *levator ani* muscle response has been used to distinguish anabolic and androgenic effects, and this may be quite different than a growth or carcass response in swine. It has already been pointed out that the feeding of 5.0 mg. of methyl testosterone per lb. of ration (Trial I) and 0.9 mg. of

11 beta-hydroxy-17 alpha-methyl testosterone per lb. or ration (Trial III) did produce a definite trend toward improved carcass leanness. Other levels of the latter hormone (0.3 and 1.2 mg. per lb. of ration) reduced the average live backfat depth.

Earlier work at this station by Beeson *et al.* (1955) and Perry *et al.* (1956) indicated that the feeding of methyl testosterone resulted in a lowered rate of gain, improved feed utilization, improved trimness in outward appearance and an increased percentage of lean cuts in the carcass. Perry *et al.* (1956) further observed a reduced daily intake of separately fed corn and an upturning of the lower tip of the vulvae of gilts fed methyl testosterone. In general Johnston *et al.* (1957) obtained nearly identical results by feeding methyl testosterone except these workers reported a decreased feed efficiency and apparently did not observe the effects upon the vulvae. Noland and Burris (1956) and Whiteker *et al.* (1957) did not obtain a reduced rate of gain from feeding methyl testosterone but did receive some effect toward improved carcass leanness. Observations of the group fed methyl testosterone (Lot II) in Trial I failed to confirm the previously observed effects upon daily gain, feed consumption, feed efficiency or outward appearance, although carcass data suggested improved carcass leanness. In Trial II the effects recorded for the lot fed stilbestrol and methyl testosterone (Lot IV) more closely resemble those obtained in previous studies; however, there was not a significant reduction in the backfat depth due to this treatment.

Woehling *et al.* (1951) and Sleeth *et al.* (1953) obtained no significant growth or carcass effects from subcutaneous administration of testosterone. Similar results were recorded with the lots receiving testosterone implants in addition to stilbestrol treatment (Lots V and VI) in Trial II.

The failure of significant growth or carcass improvement with the lots fed or implanted with stilbestrol is in agreement with the work of others (Dinusson *et al.*, 1951; Woehling *et al.*, 1951; Pearson *et al.*, 1952; Beeson *et al.*, 1955; Taylor *et al.*, 1955). Other studies have indicated that stilbestrol treatment may produce an increased growth rate either when fed singly (Braude, 1947) or fed in combination with thyroxine active agents (Braude, 1950; Barber *et al.*, 1953). Sewell *et al.* (1957) and Heitman and Clegg (1957) observed a definite growth depression when stilbestrol was administered to weanling pigs and Pearson *et al.* (1952) observed a similar response from stilbestrol implanted boars. Significant trends toward carcass leanness coupled with the reduced gains of pigs treated early in the growth period was also observed by Heitman and Clegg (1957).

Genital and mammary stimulation by DES has been previously recorded by others (Dinusson *et al.*, 1951; Pearson *et al.*, 1952; Beeson *et al.*, 1955; Taylor *et al.*, 1955; Sewell *et al.*, 1957; Heitman and Clegg, 1957). However, the degree and rapidity of stimulation observed in this study was not as great as has been reported. The reason for this lessened effect could have

been due to the lower levels of treatment or to the simultaneous treatment with testosterone.

Various data recorded in Trial II indicated that the maximum response from the stilbestrol treatments employed occurred from the implant, whereas the maximum effect from the testosterone treatments was with orally administered methyl testosterone.

Bratzler *et al.* (1954) also reported growth and carcass data very similar to the findings with the barrow and boar lots (Lots II and III) in Trial II. It had been hoped that a more marked testicular inhibition would be produced. Earlier work by Stob (1956) showed that in weanling pigs following DES-fed cattle that the testes remained undeveloped. Pearson *et al.* (1952) did not record any marked reduction in the sexual activity of boars implanted with stilbestrol. The studies of Wallace (1949) similarly failed to reveal any decrease in total sperm output following stilbestrol implantation of boars.

The highly significant increase in the iodine number of the depot fat of methyl testosterone treated pigs (Lot II) in Trial I was indicative of an increased deposition of unsaturated fats. These results tend to agree with the work of Bratzler *et al.* (1954) who found that boars developed softer carcasses than barrows. Deans *et al.* (1953) did not find any change in the iodine numbers of fat from cattle implanted with a progesterone-stilbestrol combination.

Summary

Three trials involving a total of 180 weanling pigs were conducted to determine the effects of various testosterone analogs, combinations of testosterone and stilbestrol and late castration upon the growth rate and carcass quality of swine. Oral administration of testosterone analogs (methyl testosterone, 9 alpha-fluoro-11 beta-hydroxy-17 alpha-methyl testosterone, 17 ethyl-19 nortestosterone and 11 beta-hydroxy-17 alpha-methyl testosterone) had no significant effect upon the growth rate or carcass quality of growing-finishing swine. However, the feeding of 5.0 mg. of methyl testosterone and 0.3, 0.9, 1.2 and 1.8 mg. of 11 beta-hydroxy-17 alpha-methyl testosterone per lb. of ration did produce effects suggesting improved carcass leanness. The feeding of methyl testosterone significantly increased the iodine number of the extracted fat from the edible portion of the carcass.

Boars fed stilbestrol gained faster prior to castration at 172 lb. average liveweight than barrows fed stilbestrol but had a lower accumulative average daily gain than stilbestrol fed barrows 10 days following castration. Neither of these differences were significant. Stilbestrol-fed boars consumed less corn per day, gained more efficiently and had significantly less live backfat depth at the last rib, last lumbar and average measure-

ments when compared separately with stilbestrol-fed barrows. A marked atrophy of the testes of boars fed stilbestrol did not occur.

Various combinations of feeding and implanting stilbestrol and testosterone had no significant effect upon growth rate or live backfat depth of growing-finishing swine.

Genital and/or mammary stimulation occurred in DES-treated gilts and barrows. Maximum stimulation occurred in the lot implanted with stilbestrol and testosterone propionate although definite enlargement was not observed until at least the end of the second week of treatment. Oral administration of methyl testosterone with stilbestrol caused a turning up of the lower tip of the vulva and an appearance of improved trimness in one trial but no stimulation when fed alone in another. Other treatments caused no recognizable side effects. Sex differences and interaction with treatments were also noted.

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THE EFFECTIVENESS OF THREE DIFFERENT METHODS OF IRON ADMINISTRATION TO YOUNG PIGS [1]

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NUTRITIONAL or iron-deficiency anemia long has been a paramount concern in the swine industry. McGowan and Crichton (1923) and Doyle *et al.* (1927) described the nature and course of the disease as well as the gross and microscopic pathology. Close confinement, early farrowing, adverse climatic conditions and heavily infested parasitic areas are contributing factors to an environment in which anemia develops very readily (Kernkamp, 1932; Moe *et al.*, 1935). The onset of secondary infections in anemic individuals is often inevitable because of the animal's low resistance to pathogens. (Bullard, 1931; Craig, 1930).

Doyle *et al.* (1927) noted a low incidence of anemia in litters farrowed outdoors and that secondary infections were minor. Doyle (1932), Kernkamp (1935) and others also found that access to soil greatly reduced anemia. Hart *et al.* (1929), Doyle (1932) and Kernkamp (1935) found that oral treatments of iron and copper salts provided the missing factor in hemoglobin production, thus minimizing the anemic condition. These methods of treatment are still employed by some hog raisers.

Most of the oral treatments must be repeated, and providing for ingestion of the correct dosage is often difficult. The intestinal mucosa represents a block in the absorption of iron (Hahn *et al.*, 1945; Granick, 1946).

London and Twigg, as cited by McDonald *et al.* (1955), developed an iron-dextran complex for the treatment of hypochromic anemia in man. Subsequently, it was used in preventing and curing anemia in pigs (Barber *et al.*, 1955; Brownlie, 1955; McDonald *et al.* 1955). This development presents a method of by-passing the mucosal barrier and gives greater assurance of correct dosage for each pig.

It was considered desirable to compare the relative preventive and curative qualities of the injectable type of treatment with two of the commonly used oral methods.

Materials and Methods

Poland China and Chester White dams were placed in concrete floored farrowing pens approximately one week before farrowing with no access to soil and were hand-fed twice daily. Straw was used for bedding. The pigs were divided into two test groups. Individuals in Group I were put

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
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on experiment at 5 days of age and those in Group II were put on at 15 days of age. Thirty-six males and 26 females were used in the two groups. Groups I and II were each divided into four treatment groups as follows.

Group I: C_1—received no iron treatment (control); P_1—received one pill at 5 days of age and one pill at 19 days of age. Each tablet contained 287.6 mg. of reduced iron; S_1—received 3 ml. of a liquid iron citrate orally at 5 days of age and an additional 3 ml. at 19 days of age. Each 3-ml. dosage contained approximately 33 mg. of iron; I_1—received one 2-ml. injection of an iron-dextran compound at 5 days of age only. Each ml. contained about 50 mg. of iron.

Group II: C_2—same as C_1 (control); P_2—same as P_1 except the time of treatment was at 15 and 29 days of age; S_2—same as S_1 except the time of treatment was at 15 and 29 days of age; I_2—same as treatment I_1 except the single injection was at 15 days of age.

Commercial products were used [3] and all pigs received dosage as recommended, except the time interval was held constant for the two oral methods.

A random order of the eight treatments was established and pigs within litters were assigned to a treatment without regard to sex or weight. In most cases litters contained more than eight pigs; therefore, it was usually possible to place littermates on each of the four treatments in Group I and on each of the four treatments in Group II. This seemed to be justified on the basis of a report by Venn *et al.* (1947) that the percentage of iron in littermates at birth is relatively constant. All pigs in Group I were blood sampled at 5 days of age and the appropriate treatments applied. Subsequent blood samples were taken at one-week intervals up to 26 days. At the third bleeding (19 days), Lots P_1 and S_1 received a second treatment. All pigs in Group II were bled and treated in an identical manner as those in Group I except that the experimental period began at 15 days of age and continued to 36 days of age.

Creep feed was not provided for the pigs, therefore the experimental materials and the dam's milk provided the only source of iron with the exception of spilled feed and the dam's feces. These latter two sources of iron were considered negligible, especially prior to the time when the pigs were approximately 3 weeks of age.

The blood samples (2.5 ml. obtained from anterior vena cava) were drawn into sterile tubes containing a dried ammonium and potassium oxalate solution and the hemoglobin content determined within 2 hours by the Photovolt (Lumetron) Photoelectric Hemoglobinometer: Model 10. Hematocrit values were determined in all cases, but due to their close relationship to hemoglobin levels they are omitted in this report.

[3] Ferro-Coppergen, manufactured by Norden Laboratories of Lincoln, Nebraska was used for the liquid treatment. Ferrico-Tablet, manufactured by Jensen-Salsbury Laboratories of Kansas City, Missouri was used for the pill treatment. The injectable treatment was an iron-dextran compound under the trade-name Armadexan of which a portion was kindly supplied by Armour Laboratories, Chicago, Illinois.

Results and Discussion

Preventive. The means for all four lots in Group I approximated 7.0 gm. of hemoglobin per 100 ml. of blood at the fifth day of life. The hemoglobin level in the control pigs decreased from 7.54 gm. at 5 days of age to 4.51 gm. at 19 days and 4.54 gm. at 26 days of age. The mean weight at 26 days for the control lot was 11.1 lb. One pig in Lot C_1 died and post-mortem examination indicated typical pathological lesions of anemia. Following treatment, Lots P_1 and I_1 increased to 7.89 and 8.93 gm. respectively by 12 days of age, but Lot P_1 decreased to 6.88 at 19 days (at which time a second pill treatment was administered) and was 7.05 at 26 days of age. Thus the single injection at 5 days of age (Lot I_1) produced hemoglobin levels at 19 and 26 days of age that were equal to or superior to those maintained by the group receiving an iron pill at 5 and 19 days of age (Lot P_1). In both of these lots, the pigs appeared healthy and vigorous and no deaths occurred. The 26-day mean weight for Lot I_1 was 15.3 lb. and for Lot P_1 was 15.1 lb.

In Lot S_1 the hemoglobin content declined to 3.33 at 19 days and had increased to 3.86 at 26 days. The iron in the liquid treatment was present as ferric iron and that in the pill as ferrous (Hahn *et al.,* 1945). Two pigs in Lot S_1 died during the experiment and post-mortem examination showed the pathological lesions of anemia as described by McGowan and Crichton (1923). The surviving pigs in Lot S_1 were very pallid in appearance and a high incidence of scouring was noted. The 26 day mean weight was 12.4 lb.

Curative. Animals in all four lots of Group II were anemic in appearance at 15 days of age and many were scouring. The hemoglobin content of Lot C_2 continued to decline from the level of 4.40 gm. at 15 days to 3.88 gm. at 29 days of age but had increased to 4.47 at 36 days to show a "spontaneous" recovery. The rise in hemoglobin content of the blood was not evident by physical characteristics. In Lots P_2 and I_2 scouring and the anemia syndrome disappeared within 7 days after treatment.

It can be noted in table 1 that the response at 15 days is very similar to the response at 5 days of age with the exception that Lot S_2 responded to treatment. The improvement in the hemoglobin level was accompanied by an improvement in the physical appearance in Lot S_2 but not to the same extent observed in Lots P_2 and I_2. At the end of the trial some pigs in Lot S_2 were still pallid, whereas others in the lot appeared healthy. Lot S_2 increased 0.92 gm. during the week following treatment at 15 days of age compared to a 3.30-gm. increase in Lot I_2.

The "spontaneous" recovery which began at about 29 days in Lot C_2 was undoubtedly due to iron intake from the dam's feed. The pigs in this lot were inferior in appearance at the end of the experiment. One pig died at 32 days of age and exhibited characteristic pathological lesions of anemia at autopsy (McGowan and Crichton, 1923).

Table I indicates that a single injection at 15 days of age (Lot I_2) did not result in as high a hemoglobin level as a single injection of the same quantity at 5 days of age, but the percent increase in hemoglobin level at 5 days was only 28.8% while at 15 days it was 73.6%.

The difference in the response of Lots S_1 and S_2 is of interest. In Lot S_1 no response was seen until the second treatment at 19 days of age. In Lot S_2 the response, although small, was evident within one week after the initial treatment. This may be an indication of the relative permea-

TABLE 1. EFFECTS OF THREE METHODS OF IRON ADMINISTRATION ON HEMOGLOBIN LEVELS IN PIGS AT DIFFERENT AGES

Group	Method of treatment	Number of pigs	Age, days				
			5	12	19	26	26
			Hemoglobin level, gm. %				Weight
I	None (C_1)[a]	7	7.54	6.16	4.51	4.54	11.1
	Liquid (S_1)	7	6.93	5.04	3.33	3.86	12.4
	Pill (P_1)	8	6.74	7.89	6.88	7.05	15.1
	Injection (I_1)	7	6.93	8.93	8.32	8.36	15.3
			Age, days				
			15	22	29	36	36
			Hemoglobin level, gm. %				Weight
II	None (C_2)	7	4.40	3.89	3.88	4.47	15.1
	Liquid (S_2)	8	4.65	4.83	5.57	5.80	17.5
	Pill (P_2)	8	5.22	6.74	7.00	7.01	18.5
	Injection (I_2)	9	4.48	7.78	7.89	7.99	19.1

[a] See text for explanation of treatment group symbols.

bility to non-reduced iron of the mucosal lining of the gut in varying degrees of anemia.

No significant difference in weight was found among the treatments within Group I or Group II. The relationships of pig weight and hemoglobin level patterns between Lots I_1 and P_1 in Group I and between Lots I_2 and P_2 in Groups II, indicate the pill method of administration was beneficial, although tending to be slightly inferior to the injection method. It should be noted that the injections were administered only once, whereas the pills were administered a second time 2 weeks later.

Summary

The hemoglobin level in pigs injected with an iron-dextran solution at 5 days of age increased from 6.93 gm. per 100 ml. at that time to 8.36 at 26 days of age. Pigs receiving the pill treatment increased from 6.74 at 5 days of age to 7.05 at 26 days of age. Hemoglobin levels in untreated

control pigs decreased from 7.54 at 5 days of age to 4.51 at 19 days and 4.54 at 26 days. In pigs receiving a liquid treatment orally, levels decreased from 6.93 to 3.33 and 3.86 during the same period.

Pigs injected with iron-dextran at 15 days of age showed an increase in hemoglobin from 4.48 to 7.99 at 36 days of age while those treated with pills increased from 5.22 to 7.01 during the same period. The hemoglobin levels in untreated pigs were 4.40, 3.89, 3.88 and 4.47 at weekly intervals from 15 days to 36 days of age, whereas levels in those receiving a liquid treatment orally were 4.65, 4.83, 5.57 and 5.80 at the same periods.

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THE EFFECT OF PRE-PARTUM INTRAMUSCULAR IRON TREATMENT OF DAMS ON LITTER HEMOGLOBIN LEVELS [1]

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NUTRITIONAL anemia in suckling pigs plagues hog raisers each farrowing season. However, there has been considerable advancement in preventing or curing anemia since it was first diagnosed by McGowan and Crichton (1923). Subsequent to the finding of successful oral treatments for anemic pigs, Hart *et al.* (1929), McGowan and Crichton (1923), and Venn *et al.* (1947) and others attempted to maintain normal hemoglobin levels in pigs by oral administration of large doses of iron salts to the dams. The attempts were made on the basis that, firstly, increased iron intake by the dam during late gestation would increase the iron stores in the fetus which would serve as reserve during the critical suckling period and, secondly, that increased iron intake by the dam during lactation would increase the iron content of the milk, thereby providing a prophylactic source of iron until the pig was old enough to eat solid feeds. Positive results were not obtained. Elvehjem *et al.* (1926) showed that iron content of cow's and goat's milk could not be appreciably increased by additional iron intake in the feed. Hahn *et al.* (1945) and Granick (1946) and others cited the intestinal mucosa as a barrier to iron absorption except in severe anemia. Thus, failure to increase hemoglobin levels in the pig by oral treatment of the dam during the prenatal and neonatal period has meant that oral administration of iron to the newborn pig has been the most satisfactory solution to the anemia problem. More recently a successful method of treating anemia with an iron-dextran compound injected into the skeletal muscle has been reported (Barber *et al.*, 1955; Brownlie, 1955; McDonald *et al.*, 1955; Kernkamp, 1957; Rydberg *et al.*, 1959).

Treatment intramuscularly without concern of the "mucosal barrier" raises the question as to whether or not treatment of the dam in late gestation would alter the hemoglobin pattern for pigs farrowed in an anemia-producing environment as described by Kernkamp (1932).

The purpose of this study was to observe hemoglobin levels of pigs at birth and their response to iron treatment after the dams had been injected with an iron-dextran compound [3] at either 2 or 4 weeks prior to the anticipated farrowing date.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
[2] Departments of Animal Husbandry and Veterinary Science.
[3] The injectable treatment was an iron-dextran compound manufactured by Armour Veterinary Technical Laboratories, Chicago, Illinois.

Materials and Methods

The Poland China and Chester White dams selected for this experiment farrowed in late April and early May of 1957. They were housed out-of-doors all winter in Wisconsin Colony Houses and were fed on an open concrete platform. The weather became particularly mild in late March so that loose soil was readily accessible in the exercise lot. The dams consisted of 10 first litter gilts and 10 multiparous sows. The dams were assigned randomly within each age group to one of three pre-farrowing treatment groups. The dam groups either were given no treatment or were injected intramuscularly with 10 ml. of an iron-dextran solution (50 mg. iron per ml.) at 2 weeks or 4 weeks prior to the anticipated farrowing date. The gilt groups are referred to as G_0, G_2, or G_4 as applicable whereas the sow groups are designated as S_0, S_2, or S_4. (The subscript $_0$ indicates no treatment, whereas $_2$ or $_4$ as a subscript indicates treatment at either 2 or 4 weeks before farrowing.) Approximately one week before farrowing, the dams were moved into concrete floored farrowing pens bedded with straw.

The 10 gilts farrowed 36 male and 42 female pigs alive and the 10 sows produced 45 males and 44 females alive. Within sow and gilt groups and within litters, pigs were assigned at random to one of three pig treatments: (1) No treatment; (2) an intramuscular injection of 2 ml. or (3) an intramuscular injection of 3 ml. of the iron-dextran solution at the fourth day of age (50 mg. of iron per ml. of solution). The pig-treatment lots are designated by using the age-treatment group designation of the dam (G_0, S_2, etc.) plus a second number in the subscript to designate the treatment received by the individual pig (e.g., G_{22} refers to pigs from the G_2 group of gilts that are treated with 2 ml. of the iron-dextran on the fourth day of age. G_{23} refers to pigs from the same gilt group, but treated with 3 ml. of the solution).

All pigs were bled within 12 hours after birth, again at 4 days of age, and weekly thereafter until 25 days of age. The hemoglobin level of the pigs at the initial bleeding was used to evaluate the effects of the pre-farrowing treatment of the dam on birth hemoglobin, while the change in hemoglobin levels of the control pigs in each litter (pigs receiving no treatment) during the experimental period served as an indication of the residual effects due to dam treatment.

The 2-ml. injection to the pigs was the commerically prescribed dosage level. The injection of 3 ml. was to ascertain if there was an advantage from the additional ml. or if there were indications of toxicity at the higher dosage level.

The single injection at 4 days of age and the dam's milk provided the only source of iron with the exception of spilled feed from the dam's feeding unit and the dam's feces. These latter two sources of iron were

considered negligible, especially prior to the time the pigs were 3 weeks of age. The sows were hand-fed twice daily.

The hemoglobin content was determined by an immediate analysis of a drop of blood from the marginal vein of the ear by the Photovolt (Lumetron) Photoelectric Hemoglobinometer, Model 10. The statistical analysis performed on these data followed the method described by Snedecor (1956) for disproportionate subclass numbers.

TABLE 1. THE EFFECTS OF PREPARTUM TREATMENT OF THE DAM AND POSTPARTUM TREATMENT OF THE PIGS ON PIG HEMOGLOBIN LEVELS AT DIFFERENT AGES

Dam age group	Time of dam treatment	Pig treatment group		Age, days				
				0[b]	4	11	18	25
Gilts	No treatment (G_0)[a]	Control	(G_{00})[a]	10.39[c]	7.19	4.88	4.38	4.72 (12)[d]
		2 ml.	(G_{02})		7.10	8.35	9.19	9.04 (7)
		3 ml.	(G_{03})		7.66	8.20	9.26	10.12 (10)
	Two weeks prepartum (G_2)	Control	(G_{20})	9.05[c]	6.82	5.64	6.17	7.60 (5)
		2 ml.	(G_{22})		6.74	9.48	10.55	9.59 (5)
		3 ml.	(G_{23})		6.62	8.67	10.51	10.86 (5)
	Four weeks prepartum (G_4)	Control	(G_{40})	9.99[c]	6.96	4.19	3.01	3.63 (7)
		2 ml.	(G_{42})		6.69	8.07	8.84	9.32 (9)
		3 ml.	(G_{43})		6.95	8.73	10.04	10.31 (8)
Sows	No treatment (S_0)	Control	(S_{00})	8.73[c]	6.33	4.42	4.51	5.28 (8)
		2 ml.	(S_{02})		6.49	9.07	9.30	9.36 (8)
		3 ml.	(S_{03})		6.29	8.70	9.93	10.11 (6)
	Two weeks prepartum (S_2)	Control	(S_{20})	10.18[c]	7.55	5.75	6.08	6.06 (8)
		2 ml.	(S_{22})		7.63	8.28	8.48	8.28 (7)
		3 ml.	(S_{23})		8.10	9.17	10.07	11.02 (9)
	Four weeks prepartum (S_4)	Control	(S_{40})	8.89[c]	6.05	4.21	3.56	4.92 (7)
		2 ml.	(S_{42})		6.21	7.07	7.30	8.02 (5)
		3 ml.	(S_{43})		6.53	8.55	9.23	9.97 (6)

[a] See text for explanation of the treatment group symbols.
[b] Samples for the zero group were taken during the first 12 hours post partum.
[c] These values represent the mean of all pigs in the respective dam-treatment group at birth.
[d] The number in parenthesis indicates the number of pigs completing the experiment in each group.

Results and Discussion

The initial hemoglobin levels of the pigs produced by gilts were significantly higher than those from sows; therefore, the data for gilt litters were analyzed separately from the sows. The mean 12-hour hemoglobin content for pigs from Groups G_0, G_2 and G_4 differed significantly ($P<0.05$) (table 1) as did the means of Groups S_0, S_2 and S_4 ($P<0.01$). At 4 days of age Groups G_0, G_2 and G_4 did not differ but the means of pigs from S_0, S_2 and S_4 continued to differ significantly ($P<0.01$). There were no differences in the rate of decline among the lots from either gilts or sows; however, the pigs in the G_2 group decreased about 25% less than the G_0 and G_4 groups from 12 hours to 4 days of age.

Since treatment began with pigs 4 days of age, further comparison of the effect of dam treatment are restricted mostly to the control lots (G_{00},

G_{20} and G_{40} for gilts, and S_{00}, S_{20} and S_{40} for sows). At 11 days of age the pigs from Lot G_{20} had a hemoglobin level of 5.64 gm., whereas those from Lots G_{00} and G_{40} were 4.88 and 4.19, respectively. This difference approached significance and indicates a more available reserve of iron in pigs from dams injected 2 weeks prior to farrowing (G_{20}). This tendency is supported further by the higher hemoglobin level in Lot G_{20} at 18 days of age ($P<0.05$) and at 25 days of age ($P<0.01$).

Sows injected 2 weeks prepartum not only produced pigs with a higher hemoglobin level at birth and at 11 days of age ($P<0.05$) but at 18 and 25 days of age also ($P<0.01$). In both dam groups injected at 2 weeks prepartum (G_{20} and S_{20}), there was a higher minimum hemoglobin level and their "spontaneous" recovery began sooner than for pigs from untreated dams or dams treated 4 weeks prepartum. The response of pigs from gilts treated 2 weeks prepartum was similar to the response of pigs from sows. Thus, the response of the hemoglobin level in pigs from the two dam groups was consistent.

It appears that when the pigs became anemic enough to stimulate increased production of red blood cells and hemoglobin, the hemopoietic centers in pigs from Lots G_{20} and S_{20} had a greater reserve of iron available than did the hemopoietic centers in pigs from dams not injected (G_{00} and S_{00}) or pigs from dams injected 4 weeks prior to farrowing (G_{40} and S_{40}). With the exception of minute quantities of iron in the dam's milk, these pigs had no source of iron except what they obtained in prenatal life and therefore the hemopoietic centers, post-partum, would have to rely on the iron reserves in the pig at birth. This reserve of iron must have been present in the body during the hemoglobin decline that occurred during the first week post-partum; thus, it appears that the hemopoietic centers may not become hyper-active until the hemoglobin content has reached a certain minimum level which then triggers an activating mechanism associated with these centers.

The lack of a difference between the hemoglobin levels of pigs from untreated dams and pigs from dams treated 4 weeks before farrowing indicates that the 4-week treatment was probably too early in gestation. There were no undesirable side effects noted as a result of treating pigs farrowed by dams treated prepartum.

The mean weight of all untreated pigs from gilts (G_{X0}) at 25 days was 10.54, compared to 12.59 for all pigs injected with 2 ml.(G_{X2}) and 12.75 lb. for all pigs injected with 3 ml.(G_{X3}) ($P<0.01$). The relationship of 25-day mean weights of pigs in the corresponding lots from sows were similar to those from gilts, the S_{X0} lot averaging 13.6, the S_{X2} lot, 16.5, and the S_{X3} lot, 15.4 lb. (An $_X$ in the subscript indicates that all dam groups are combined.)

Three ml. of iron-dextran did not produce a significantly higher hemoglobin level or rate of gain than 2 ml. Neither did it show any undesirable side effects.

There were no differences in average birth weights among the groups. Litter size was not affected by dam treatment. It will be necessary to obtain more information on the placental transfer and metabolism of iron before the exact benefit to the fetus, resulting from dam therapy with iron, can be understood. As shown in table 1, treatment of individual pigs with either 2 ml. or 3 ml. of the iron-dextran solution was beneficial in maintaining the hemoglobin level and further documents the results of a previous report (Rydberg *et al.*, 1959).

Summary

Dams treated 2 weeks before farrowing with an intra-muscular injection of 10 ml. of an iron-dextran solution produced pigs which maintained a higher hemoglobin level during the critical post-partum period than did pigs from untreated dams or from dams treated 4 weeks prior to farrowing. Differences in hemoglobin levels in the pigs from untreated dams and in pigs from dams treated 4 weeks before farrowing were not significant.

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SIGNIFICANT RELATIONSHIPS IN PORK CARCASS EVALUATION [1, 2]

I. Lean Cuts as Criteria for Live Hog Value

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THE appraisal of hogs based on live hog scores or visual appearance may be misleading (Bogart *et al.*, 1940; Bruner, 1952; Peterson, 1952). Wiley *et al.* (1951) pointed out that differences in the relative proportions and qualities of the wholesale cuts can be determined more accurately from the carcass than from the live hog. Many other workers have reported the same general conclusions. If the evaluation is to be made from the carcass and its cuts, it is essential that pertinent and accurate measurements be taken.

This paper presents the results of an experiment to determine the relationship of the four lean cuts, five primal cuts and total lean to specific measurements obtained on the live hog and carcass. The relationship of live hog and carcass measurements to fat and fat cuts will be reported in a subsequent paper.

Materials and Methods

For this study 207 hogs were slaughtered during the autumn of 1952 and the spring of 1953. They consisted of 122 Landrace-Poland crosses, 76 Hampshires and 9 Durocs. Backfat probe measurements were taken from 116 of the hogs the day before slaughter. The probes were inserted 1.5 in. off the mid-line at the 5th-6th thoracic (shoulder probe), the 4th-5th lumbar (hip probe) and 2nd-3rd sacral vertebra (ham probe). Hogs attaining a weight of 205 to 215 lb. were weighed, fasted 24 hours, re-weighed and immediately slaughtered. All hogs were dressed packer style; head off, leaf fat attached and hams unfaced. Carcasses were weighed individually once within 2 hours after sticking and again after chilling 24 hours at 32-35° F. Carcass measurements were taken after chilling and as the carcasses were processed.

The carcass measurements included, carcass backfat thickness, carcass length, ham length, ham circumference, depth and width of the lean area of the ham butt end, depth and width of the longissimus dorsi (loin eye) muscle, and the depth and width of psoas major (tenderloin) muscle.

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The carcass backfat measurements were taken at the first rib, last rib and last lumbar vertebra. Carcass length was measured from the anterior edge of the aitch bone to the anterior edge of the first rib. Ham length was measured from the distal edge of the aitch bone to the tibia projection (medial malleolus). Ham circumference was measured at the midpoint of the ham length. Depth of lean area of the ham butt end was measured from the ventral edge of the exposed cut ilium to the dorsal edge of the subcutaneous fat, (a vertical line when the untrimmed ham is placed skin side down on a table). Width of lean area of the ham butt end was measured on a plane parallel to the table top across the ventral edge of the ilium. The product of the depth x width measurement was referred to as the ham lean cross section area ($mm.^2$). The depth of the *longissimus dorsi* (loin eye) muscle was measured immediately posterior to the last rib. The width of loin eye was measured at a right angle to the depth measurement. The product of these two measurements was referred to as the loin eye cross section area ($mm.^2$). The depth and width of *psoas major* (tenderloin) muscle was measured on the butt end of the untrimmed ham. The product of these two measurements constituted the tenderloin cross section area ($mm.^2$). The carcasses were broken down into wholesale cuts and each cut trimmed as outlined in the Proceedings of the 1952 Reciprocal Meat Conference. Weights were recorded to the nearest 0.1 lb. and linear measurements to the nearest millimeter.

The yield of the carcass and wholesale cuts were based on the empty liveweight and the chilled carcass weight, respectively. The empty liveweight was determined by subtracting the weight of the digestive tract contents (fill) from the 24-hour-fasted weight (shrunk weight). The digestive tract included the small and large intestines, stomach, bladder, spleen and adhering intestinal fat. The tract was weighed immediately following evisceration. The intestines and stomach contents were stripped out, flushed with water, drained and re-weighed. This weight constituted the empty digestive tract weight. The difference between the full and empty digestive tract weight is "fill".

Results and Discussion

Four Lean Cuts. Each of the three live hog backfat probe measurements was correlated with the yield of the four lean cuts (ham, loin, Boston butt and picnic). The shoulder probe measurement showed the least relationship, the hip probe measurement the greatest relationship, and the ham probe measurement was intermediate in its relationship to the four lean cuts, table 1. The relative magnitude of the relationship between each of the three backfat probes and the four lean cuts can be illustrated by comparing "r^2", the coefficient of determination. The coefficient of determination for the shoulder probe is 0.04 ($.21^2$), hip probe 0.18 and ham probe 0.12. This is the proportion of the variance of the

four lean cuts that can be explained by each probe measurement. The hip probe accounts for more than 4.5 times as much of the variance in the four lean cuts as the shoulder probe and approximately 50% more than the ham probe.

Each of the three probes was also used as an independent variable in a multiple correlation to determine their combined relationship to the four lean cuts. The coefficient of multiple correlation was 0.45. The calculated regression equation was:

Yield of four lean cuts = 44.27 + 0.028 x shoulder probe (mm.) —0.173 x hip probe (mm.) —0.060 x ham probe (mm.).

TABLE 1. CORRELATIONS OF THE FOUR LEAN CUTS AND FIVE PRIMAL CUTS WITH CARCASS MEASUREMENTS AND YIELDS

Items	Four lean cuts[1]	Number in sample	Five primal cuts[2]	Number in sample
Shoulder probe, mm.	−.21*	108	−.15	108
Hip probe, mm.	−.43**	108	−.32**	108
Ham probe, mm.	−.35**	108	−.23*	108
Probes, av. of three, mm.	−.44**	116	−.35**	116
Carcass backfat measurements av. of three, mm.	—	—	−.27**	189
Loin, percent liveweight	+.74**	108	+.69**	108
Ham, percent liveweight	+.73**	108	+.37**	108
Shoulder, percent liveweight	+.70**	108	+.55**	108
Cooler shrink, %	+.58**	108	—	—
Ham trim fat, lb.	−.46**	108	−.35**	108
Loin eye cross section area, mm.^{2}	+.57**	204	+.38**	186
Tenderloin cross section area, mm.^{2}	+.19*	120	+.18*	120

[1] Ham, loin, picnic, Boston butt.
[2] Ham, loin, picnic, Boston butt, belly.
** Probability of chance occurrence <.01.
* Probability of chance occurrence <.05.

When the mean value of three live hog probes was correlated with the percent yield of the four lean cuts, the correlation was —.44. In no case were the three probes as highly related to the four lean cuts as has been reported by Hazel and Kline (1952).

The higher correlations between loin, ham or shoulder and the four lean cuts, r= +.74, +.73, +.70, respectively, were influenced by the part-whole relationship. Nevertheless, the magnitude of these correlations indicate that any one of these three cuts could be employed in a regression equation for estimating the yield of the four lean cuts.

The loss in carcass weight during the 24-hour chill period was directly associated with the yield of four lean cuts, r=+.58. This was expected since a greater yield of lean is associated with thinner (less highly finished) carcasses which normally lose more moisture during chilling.

The ham trim fat which was removed from hams during processing to skinned hams was inversely correlated with the yield of four lean cuts, r=—.46. A negative "r" would be expected between these two variables since an increase in ham trim fat is associated with an increase in carcass trim fat which is in turn inversely associated with the yield of lean.

The loin eye cross section area was highly associated with the yield of four lean cuts, r=+.57. A correlation of approximately this magnitude between these two variables was expected, since the four lean cuts are composed of fat, lean, bone and skin. McMeekan (1941) illustrated that the loin eye cross section area was directly correlated (r=+.84) with the weight of total "separable" lean from the carcass.

Five Primal Cuts. The relationship of each of the three live hog backfat probe measurements to the yield of the five primal cuts (four lean cuts plus the belly) was also determined. These correlations were all negative. Ranked in order of magnitude, they were hip probe, —.32; ham probe, —.23; and shoulder probe, —.15. The average of the probe measurements when correlated with the five primal cuts resulted in an "r" of —.35. This average divided by the carcass length and then correlated with the five primal cuts increased the "r" to —.51. This means 13.76% more, or twice as much, of the variation in the five primal cuts was accounted for by considering carcass length with the live hog probes.

The trimming fat from the hams was correlated with the five primal cuts, r=—.35. This correlation can be expected to vary, depending on the consistency in trimming the ham. The cross section area ($mm.^2$) of the loin eye and tenderloin increased as the yield of the five primal cuts increased. The correlations were +.38 and +.18, respectively. The correlation between the five primal cuts and the hams, loins, and shoulders are given in table 1. All of these correlations are somewhat lower than those with the four lean cuts. This is due to the inclusion of the belly which is considered to be an intermediate cut with regard to lean-fat ratio.

Four Lean Cuts Plus Lean Trim. The four lean cuts plus lean trim (hams, loins, picnics, Boston butts, and lean trimmings) as a percent of the liveweight were correlated with the cross section area of the loin eye (r=+.60) and to a lesser extent with the ham lean cross section area of the ham (r=+.46; see table 2).

The cross section areas of the loin eye and the ham lean were used as independent variables in a multiple correlation to determine their combined relationship to the yield of four lean cuts plus lean trim. The coefficient of multiple correlation was 0.652. When length of carcass was included as the third independent variable the coefficient of multiple correlation increased to 0.654, indicating that length of carcass had very little relationship to the yield of the four lean cuts plus lean trim. When

the ham index (ham circumference x 100÷ham length) was used in lieu of the ham lean area in this multiple correlation, the coefficient of multiple correlation decreased to 0.538. This decrease in the coefficient of multiple correlation coupled with the nonsignificant correlation ($r = +.13$) between the ham index and yield of four lean cuts plus lean trim strongly suggests that the ham index has little value as a means of predicting the yield of four lean cuts plus lean trim from a carcass.

The intercorrelations indicated that ham lean cross section area increased with an increase in loin eye cross section area, $r = +.36$, $N = 186$, while both variables decreased as carcass length increased, $r = -.11$, $r = -.37$,

TABLE 2. CORRELATIONS OF THE YIELD OF FOUR LEAN CUTS PLUS LEAN TRIM TO OTHER MEASUREMENTS AND YIELDS

Items	Lean[1]	No. in sample
Dressing percent	$+.36^{**}$	189
Ham index	$+.13$	184
Trim fat,[2] percent liveweight	$-.62^{**}$	189
Ham lean cross section area, mm.[2]	$+.46^{**}$	186
Loin eye cross section area, mm.[2]	$+.60^{**}$	186
Carcass length, mm.	$-.18^{*}$	186
Tenderloin cross section area, mm.[2]	$+.19^{*}$	120

[1] Four lean cuts plus lean trim.
[2] Trim fat includes carcass trim fat, backfat and kidney fat.
** Probability of chance occurrence $<.01$.
* Probability of chance occurrence $<.05$.

respectively. These analyses suggest that in the case of these muscle areas, an increase in carcass length was at the expense (a decrease) of the cross section area of the loin and ham muscle.

No strong relationship was found between carcass length and the yield of lean. The calculated "r's" were as follows: Landrace-Poland crosses, $-.20$; Hampshires, $+.12$; and Durocs, $+.26$. When these hogs were considered as a composite group the "r" was $-.18$. These "r's" are not applicable to broad generalizations. A correlation for the same two variables was calculated on 358 hogs slaughtered in earlier studies with a resultant "r" of $+.13$. These correlations suggest that carcass length alone is a questionable index to employ for estimating the yield of lean.

The cross section areas of the loin eye and the tenderloin, respectively, were positively correlated with the yield of lean, $r = +.60$ and $+.19$. McMeekan (1941) reported a highly significant correlation, $r = +.81$, between the weight of the tenderloin and the yield of "separable" lean.

The results of this study indicate that live hog backfat probes, various carcass measurements and cuts can be employed as criteria for live hog value. The live hog backfat probe taken at the 4th-5th lumbar vertebra (hip probe) was superior to the (shoulder) 5th-6th thoracic or the

(ham) 2nd-3rd sacral vertebra probe as an index to the yield of the four lean cuts. The shoulder probe was the least important in this respect. These same conclusions apply in regard to the five primal cuts. Carcass measurements obtained from a cross section surface provide a good index of muscle development. The loin eye cross section measurement was more highly associated with the yield of lean than the ham or tenderloin (psoas) cross section area. There was little difference between the value of the yield of loin, ham or shoulder, as an index of leanness, as measured by the yield of four lean cuts. The consistency of the cutting and trimming procedure would be very important if one of these cuts was used as an index of carcass value.

The weight of ham trim fat which is removed from hams during processing to skinned hams, under a standardized trimming procedure, has utility as a tool in carcass evaluation. The advantages of the ham trim fat for this purpose is evident since many carcass processing establishments normally trim hams before curing or further processing. The ham index (ham circumference x 100÷ham length was not significantly associated with measures of carcass value.

Carcass length was not strongly associated with the yield of lean. The relationship of carcass length with the yield of lean appears to vary directly with the muscular development of the hog as long as "growth" (i.e., the dimensional ratios of the masculature increase) in all directions is constant. In this study, the cross section area of the loin eye and ham lean (butt end) tended to be smaller from the longer carcasses.

Summary

The 207 hogs slaughtered for this study consisted of 122 Landrace-Poland crosses, 76 Hampshires, and 9 Durocs of approximately 206 lb. liveweight.

The yield of the five primal cuts was negatively correlated with the backfat thickness measurements and the weight of ham fat trim. The loin eye cross section area, loss in carcass weight during the chill period and the yield of loin, ham or shoulder, were correlated with the yield of four lean cuts. These same variables were also significantly correlated with the yield of the five primal cuts, but to a lesser degree than with the four lean cuts.

The cross section area of the loin eye, cross section area of the ham lean and dressing percent were significantly correlated with the yield of total lean. The single variable most highly associated with the yield of total lean was the cross section area of the loin eye. This variable was also highly correlated with the yield of the four lean and five primal cuts. The correlations, in order of magnitude, between the four lean cuts and each of the live hog probes were hip probe, ham probe and shoulder probe.

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STILBESTROL FEEDING AND GROWTH HORMONE STIMULATION IN IMMATURE RUMINANTS [1, 2, 3]

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STILBESTROL (diethylstilbestrol) when administered in proper amounts to immature cattle and sheep not only stimulates rate of liveweight gain and reduces feed requirements per unit of gain but also increases nitrogen retention in these particular species of farm animals (Clegg and Cole, 1954; Story, 1955; Bell *et al.*, 1955; Campbell *et al.*, 1956; Struempler and Burroughs, 1956). Growth hormone appears to exert a somewhat similar influence upon nitrogen retention in certain other species of animals. Lee and Schaeffer (1934) were among the first to demonstrate that the administration of growth hormone increased nitrogen retention in the rat by decreasing urinary nitrogen excretion. These results were subsequently confirmed by Gordon *et al.* (1948). However, information is lacking as to whether this similarity of response of stilbestrol and growth hormone upon nitrogen retention in ruminants is directly related physiologically. It seemed plausible that since stilbestrol has estrogenic properties that it might stimulate growth hormone production in cattle and sheep, since hormones in general are sometimes known to exert chainlike influences upon one another. The purpose of this study was to determine possible physiological relationships between stilbestrol administration and accelerated growth hormone production in ruminants.

Experimental Procedure

Two types of experiments were conducted. One consisted of a nitrogen balance study with lambs with and without stilbestrol and growth hormone administration. The second type of experiment included a bio-assay study of the growth hormone content of the anterior pituitary gland of young cattle fed varying amounts of stilbestrol for long periods of time.

Two groups each of 12 wether lambs were used in two series of nitrogen balance experiments. Both experiments were carried out over a 36-day period in which nine 4-day collections were made on each lamb. A total of 216 4-day collections were made in which 24 treatments using three

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[3] The growth hormone used in this work was a gift from the Endocrinology Study Section, National Institutes of Health, Bethesda, Maryland.

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TABLE 1. EXPERIMENTAL DESIGN OF LAMB NITROGEN RETENTION EXPERIMENT

Ration treatment			No growth hormone injected			Growth hormone injected			No growth hormone injected		
Protein %	2 mg. stilbestrol	Level of energy	1st 4-days	2nd 4-days	3rd 4-days	1st 4-days	2nd 4-days	3rd 4-days	1st 4-days	2nd 4-days	3rd 4-days
8.5	..	Low	3	3	3	3	3	3	3	3	3
8.5	..	High	3	3	3	3	3	3	3	3	3
8.5	+	Low	3	3	3	3	3	3	3	3	3
8.5	+	High	3	3	3	3	3	3	3	3	3
13.0	..	Low	3	3	3	3	3	3	3	3	3
13.0	..	High	3	3	3	3	3	3	3	3	3
13.0	+	Low	3	3	3	3	3	3	3	3	3
13.0	+	High	3	3	3	3	3	3	3	3	3

lambs per treatment were triplicated with each lamb. The experimental design is illustrated in table 1. Ration variables of protein and energy were included in this design since they had previously been shown (Struempler and Burroughs, 1956) to exert influences upon the action of stilbestrol in increasing nitrogen retention. The 8.5% protein ration consisted of a mixture of the following ingredients: Cracked corn, 44, ground alfalfa hay 25, ground corncobs 15, cane molasses 15 and salt 1. The 13.5% protein ration was similar to the 8.5% ration except for the substitution of 11% soybean meal in place of an equal amount of cracked corn. Ration energy intake was altered by increasing or decreasing the amount of the respective rations fed. The total digestible nutrient (TDN) content of each ration was estimated to be 64%. The low energy level of feeding was estimated to furnish 110% of the lamb's maintenance requirements. The high-energy level of feeding was estimated to furnish 170% of the lamb's maintenance requirements. Stilbestrol when added to the ration supplied approximately 2 mg. per lamb per day.

The lambs initially weighed an average of 77 lb. Collection of excreta samples and analysis for nitrogen and dry matter were made, using conventional methods. Growth hormone injection was at the rate of 12.5 mg. of purified growth hormone preparation per lamb per day. This amount of growth hormone [5] was dissolved in 0.5 ml. of sterile saline and injected intramuscularly in a single dose.

The procedure followed in the assay studies of the anterior pituitary glands of cattle fed varying amounts of stilbestrol was essentially similar to the growth hormone assay procedure described by Russell (1955). Pituitary glands were collected from 72 beef type steers at the time of slaughter. The steers had been fed in 12 lots each of six animals for 203 days in which one-third of the cattle received no stilbestrol, one-third received 5 mg., and one-third of the cattle received 10 mg. of stilbestrol per animal daily in their feed. The cattle weighed intially about 500 lb. and were approximately 300 days of age. Their weight at the time of slaughter averaged 1000 lb. per animal (Culbertson *et al.*, 1956). The cattle feeding design was as follows:

CATTLE FEEDING DESIGN

	Medium protein (10.5%) ration		High protein (13.5%) ration	
Treatment	Low-gainers	High-gainers	Low-gainers	High-gainers
No stilbestrol	Lot 1	Lot 2	Lot 3	Lot 4
5 mg. stilbestrol	Lot 5	Lot 6	Lot 7	Lot 8
10 mg. stilbestrol	Lot 9	Lot 10	Lot 11	Lot 12

[5] The growth hormone lot No. R–50109. Somar-A, is a highly purified bovine preparation distributed by the National Institute of Health.

The pituitary glands from the cattle were frozen with dry ice (carbon dioxide) immediately following slaughter and remained in a frozen condition until prepared for assay. Preparation for assay consisted of thawing and dissecting the anterior lobe from surrounding tissue. The anterior lobe was then weighed and homogenized in saline solution (3 ml. of 0.85% saline solution per gm. tissue) using a Potter-Elvehjem type of tissue homogenizer. The resulting solution was centrifuged for 15 minutes at 500 rpm. at 830 x gravity and the decanted extract further diluted with physiological saline to a final volume of 1 gm. of tissue to 16 ml. of saline. This extract was refrigerated at 1° C. until assayed.

Hypophysectomized female rats of approximately 4 weeks of age were used as the assay animal. Following a 13-day post-operative period, three rats were randomly allotted to each lot of pituitary material to be assayed. The extracts were administered by intraperitoneal injections of 0.5 ml. of the prepared extract daily for 4 days. Twenty-four hours after the last injection the rats were sacrificed, the right tibia dissected, split with a sharp razor blade at the proximal end of the mid-sagittal plane, and stained according to the method of Russell (1955). The growth hormone content of the anterior pituitary extracts was estimated by measuring the average width of the uncalcified epiphyseal cartilage of the tibia. The desired response was to obtain a working range between 190 and 340 micra as described by Greenspan *et al.* (1949). Several preliminary bio-assay trials were conducted to determine the extraction procedure of the pituitary glands and the proper dilution of the pituitary extract necessary in meeting this criterion. Since the primary objective of the experiment was to obtain relative differences rather than quantitative determinations of growth hormone potency between the growth hormone content of the pituitaries of stilbestrol-treated steers as compared to their controls, no standard curve was established. Instead, any differences in growth hormone potency between pituitary glands were based on differences expressed in micra, in the width of the uncalcified epiphyseal cartilages of the rat tibiae.

Results

Nitrogen balance. Nitrogen retention in the lambs was increased both by stilbestrol feeding and growth hormone injection (table 2). The magnitude of the increases noted was essentially similar, averaging 48% in the case of stilbestrol and 50% in the case of growth hormone. The simultaneous administration of both materials resulted in approximately a double increase (123%) in the amount of nitrogen retained as compared to that noted when either of the two materials was added singly. Raising the percentage of protein in the ration from 8.5% to 13.0% also increased nitrogen retention by 50% or about an identical amount to that occurring from either stilbestrol or growth hormone administration. Also increasing the level of ration fed from an estimated 110% of maintenance to 170%

of maintenance increased nitrogen retention by 115%. It is obvious, therefore, that each of the four variables studied in this experiment, namely, stilbestrol, growth hormone, ration protein level and ration energy level, all exerted positive influences upon the amount of nitrogen retained by the lambs.

On the contrary, digestibility differences in ration protein and ration dry matter (table 2) were little affected by the four variables under study, aside from expected differences in apparent nitrogen digestibility with different protein intakes. Neither stilbestrol nor growth hormone exerted

TABLE 2. THE EFFECT OF GROWTH HORMONE ADMINISTRATION WITH AND WITHOUT STILBESTROL ON NITROGEN RETENTION AND DIGESTIBILITY IN LAMBS RECEIVING RATIONS HIGH AND LOW WITH RESPECT TO PROTEIN AND ENERGY

Ration treatment					
Protein %	Stilbestrol	Energy level	No growth hormone injected	Growth hormone injected	No growth hormone injected
			Nitrogen retention (gm./lamb/day)		
8.5	..	Low	0.9[a]	1.9	0.7
8.5	..	High	2.7	4.4	2.2
8.5	+	Low	1.7	1.9	0.6
8.5	+	High	4.5	5.0	3.9
13.0	..	Low	2.0	2.8	1.3
13.0	..	High	3.9	5.8	2.0
13.0	+	Low	3.4	4.0	3.0
13.0	+	High	6.0	6.8	4.6
			Apparent digestibility of ration protein (%)		
8.5	..	Low	60	60	64
8.5	..	High	60	59	61
8.5	+	Low	60	63	63
8.5	+	High	57	60	62
13.0	..	Low	75	75	75
13.0	..	High	69	73	72
13.0	+	Low	74	75	75
13.0	+	High	67	69	71
			Digestibility of ration dry matter (%)		
8.5	..	Low	74	75	76
8.5	..	High	72	71	71
8.5	+	Low	75	76	77
8.5	+	High	72	74	75
13.0	..	Low	77	77	78
13.0	..	High	71	74	72
13.0	+	Low	76	77	78
13.0	+	High	72	74	75

[a] Standard error of the mean has been computed for each value and is on file in the Animal Husbandry Department at Iowa State College, Ames.

measurable influences upon protein or dry matter digestibility. Increasing the level of feeding slightly reduced dry matter digestibility.

Growth Hormone Assay. The growth hormone index assays of the anterior pituitary glands are summarized in table 3. Although considerable variation existed in the data between lots of cattle receiving a given level of stilbestrol, nevertheless the average growth hormone values increased with each succeeding level of stilbestrol consumed by the cattle.

TABLE 3. ESTIMATION OF GROWTH HORMONE INDEX IN PITUITARY GLAND OF CATTLE FED DIFFERENT AMOUNTS OF STILBESTROL

Items	10.5% protein ration		13.6% protein ration		Average
	Low gainers	High gainers	Low gainers	High gainers	
Cattle receiving no stilbestrol					
Lot no.	1	2	3	4	
Av. weight anterior pituitary, gm.	1.40	1.05	1.30	0.96	1.18
Av. epiphysis width, microns.	255	275	277	268	
Growth hormone index (wt. x width)	357	289	360	257	316
Av. weight of cattle, lb.	909	979	1008	991	
G. H. index/lb. cattle liveweight	39	30	36	26	33
Cattle receiving 5 mg. stilbestrol					
Lot no.	5	6	7	8	
Av. wt. anterior pituitary, gm.	1.16	1.61	1.45	1.51	1.43
Av. epiphysis width, microns.	208	273	278	256	
Growth hormone index (wt. x width)	241	440	403	387	369
Av. weight of cattle, lb	960	1094	1047	1030	
G. H. index/lb. cattle liveweight	25	40	39	38	36
Cattle receiving 10 mg. stilbestrol					
Lot. no.	9	10	11	12	
Av. wt. anterior pituitary, gm.	1.57	1.55	1.39	..	1.50
Av. epiphysis width, microns.	314	240	251	..	
Growth hormone index (wt. x width)	493	372	349		405
Av. weight of cattle, lb.	1043	1097	1024	..	
G. H. index/lb. cattle liveweight	48	34	34		39

A noticeable response from stilbestrol feeding was an increase in total weight of the anterior pituitary gland. The cattle receiving 0, 5, and 10 mg. levels of stilbestrol had average anterior pituitary gland weights of 1.18, 1.43 and 1.51 gm., respectively. Somewhat smaller but substantial increases in total growth hormone activity per gland were noted with each successive level of stilbestrol fed. The total growth hormone index per pituitary gland was 316, 369 and 405 for the cattle receiving 0, 5 and 10 mg. levels of stilbestrol, respectively.

Discussion

The results obtained in this study can best be reconciled on the basis that the physiological activity of stilbestrol in bringing about increased liveweight gains and nitrogen retention in immature ruminants is mediated primarily through growth hormone stimulation. It is postulated that growth hormone secretion is increased following administration of stilbestrol to young cattle and sheep. Furthemore, continued administration of stilbestrol over long periods of time results in continued hypersecretion of growth hormone which brings about enlargement of the anterior pituitary gland much as hyperexcretion of nitrogenous products increases kidney size.

The most suggestive but by no means conclusive evidence that stilbestrol stimulates growth hormone production in lambs is the similarity in response upon nitrogen retention brought about by supplementary growth hormone and the administration of stilbestrol. In the present studies nitrogen balance was not only increased when each was given separately but the response from growth hormone was immediate when given and when withdrawn. Stilbestrol given to cattle and growth hormone administration in other species is known similarly to increase calcium and phosphorus balances as well as lower blood serum amino nitrogen (Preston, 1957).

The increased size of the anterior pituitary gland in cattle accompanying stilbestrol usage in this study is further suggestive evidence of increased secretory activity. Somewhat similar results were obtained by Clegg *et al.* (1954) and Cahill *et al.* (1956). The increased size of the anterior pituitary gland with stilbestrol feeding in the present studies would seem to have additional significance since the gland increased greater in weight (29%) than did the total tissues (liveweight 9%) of the cattle fed 10 mg. of stilbestrol daily.

Further evidence in support of stilbestrol increasing growth hormone production in cattle was the finding in this study that on an equal weight basis of the anterior pituitary gland, the growth hormone activity was essentially the same irrespective of level of stilbestrol fed. This would suggest but cannot be regarded as conclusive evidence that the residual growth hormone in the gland may be a reliable index of the total growth hormone secretory activity in the live animal. The plausibility of this assumption has been pointed out by Baird *et al.* (1952) in which he suggested that a given unit of swine pituitary tissue, regardless of age, weight or sex, secretes a constant quantity of growth hormone.

Finally, upon the assumption that the residual growth hormone in the *post mortem* anterior pituitary gland is a reliable index of growth hormone secretion in the live animal, the present studies show a progressive trend in greater growth hormone activity with increasing levels of stilbestrol. This increase when corrected for body size amounted to 9% with the 5 mg. level and 18% with the 10 mg. level. The reliability of these per-

centage figures could not be tested statistically but they are also of interest since they are of the same magnitude as the growth response often noted in cattle from stilbestrol administration.

The nitrogen balance data as presented in table 2 were analyzed in table 4 with respect to the independence or the interdependence of the four

TABLE 4. INTERRELATIONSHIPS BETWEEN VARIABLES INFLUENCING NITROGEN RETENTION IN LAMBS

Variable (s)	Av. nitrogen balance/lamb/day, gm.			Expected diff. if individual variable influences are additive
	Negative control	Plus variable (s)	Actual diff.	
Stilbestrol alone	2.6	3.8	+1.2	
Growth hormone alone	2.7	4.1	+1.4	
Protein level alone	2.5	3.8	+1.3	
Energy level alone	2.0	4.3	+2.3	
Stilbestrol plus growth hormone	2.0	4.4	+2.4	+2.6
Stilbestrol plus protein level	2.1	4.6	+2.5	+2.5
Stilbestrol plus energy level	1.6	5.1	+3.5	+3.5
Growth hormone plus protein level	2.2	4.9	+2.7	+2.7
Growth hormone plus energy level	1.7	5.5	+3.8	+3.7
Protein level plus energy level	1.3	4.9	+3.6	+3.6
Stilbestrol+growth hormone+protein	1.6	5.4	+3.8	+3.9
Stilbestrol+growth hormone+energy	1.2	5.9	+4.7	+4.9
Stilbestrol+protein+ energy	1.2	5.8	+4.6	+4.8
Growth hormone+protein +energy	1.0	6.3	+5.3	+5.0
Stilbestrol+growth hormone+protein level+energy level	0.8	6.8	+6.0	+6.2

demonstrated influential factors, namely, stilbestrol, growth hormone, protein level and energy level. Each factor appeared to behave independently since the individual influence of one factor when superimposd upon one, two or three other factors remained constant (see table 4). This independence of action of the four factors may in part be apparent rather than real. For example, even though stilbestrol and growth hormone appeared to exert independent influences upon nitrogen retention, these influences

may in reality be due to growth hormone in which the stilbestrol stimulated the lamb's anterior pituitary secretion. It would be necessary to inject higher levels of growth hormone with and without stilbestrol feeding before one could determine whether the two influences are the same or independent of one another with respect to increasing nitrogen balance.

The nitrogen balance data as presented in table 2 were analyzed further in table 5 with respect to type of ration most conducive to increased nitrogen retention by lambs either fed stilbestrol or injected with growth

TABLE 5. TYPE OF RATION MOST CONDUCIVE TO INCREASED NITROGEN RETENTION IN LAMBS RESULTING FROM EITHER STILBESTROL FEEDING OR GROWTH HORMONE INJECTION

Type of ration	Av. nitrogen balance/lamb/day, gm.		
	Negative control	Stilbestrol or growth hormone	Diff.
Stilbestrol responses			
Low-energy and low protein	1.2	1.4	0.2
High-energy and low protein	3.1	4.5	1.4
Low-energy and high protein	2.0	3.5	1.5
High-energy and high protein	3.9	5.8	1.9
Growth hormone responses			
Low-energy and low protein	1.0	1.9	0.9
High-energy and low protein	3.3	4.7	1.4
Low-energy and high protein	2.4	3.4	1.0
High-energy and high protein	4.1	6.3	2.2

hormone. The stilbestrol response was lowest, in fact, almost non-existent, on a low-protein ration fed at a low-energy intake level. Conversely, stilbestrol increased nitrogen retention 10-fold when the ration protein was increased and the ration fed in more liberal amounts. Growth hormone behaved rather similarly to stilbestrol in that it was least effective in improving nitrogen retention on a low-protein and low-energy ration and most effective on a high-protein and high-energy ration. The magnitude of the response difference (table 5) due to ration was less in the case of growth hormone as compared to that of stilbestrol.

Summary

Nitrogen balance experiments with lambs and growth hormone assay experiments in cattle were carried out to study the physiological mechanism whereby stilbestrol in proper amounts brings about accelerated liveweight gains in immature ruminants. Nitrogen balance was favorably influenced by stilbestrol, growth hormone administration and higher ration energy and protein levels. The cattle growth hormone assay results revealed a

positive relationship with the amount of stilbestrol fed. Stilbestrol feeding increased the size of the anterior pituitary, total amount of growth hormone in the anterior pituitary and the amount of growth hormone per unit of cattle weight. The results were discussed as evidence supporting the theory that the growth-stimulating influence of stilbestrol in immature ruminants is primarily mediated through growth hormone stimulation within the animal body.

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PERFORMANCE OF CROSSBRED BEEF CATTLE IN THE GULF COAST REGION [1]

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THE Gulf Coast Region of the United States has been a leading area in crossbreeding beef cattle for many years. The extensive use of this method of breeding has resulted because of the inability of the English beef breeds to thrive under the difficult climatic conditions. Early importations of Brahman cattle demonstrated the ability of these cattle to tolerate the heat and humidity characteristic of this region, and also their ability to improve the native stock in crosses. The early work with Brahman cattle was conducted primarily by private breeders so the results were not available for study and analysis.

Lush *et al.* (1930) reported the results of 9 years of crossbreeding work under Texas range conditions. They found that Brahman-Hereford cows raised considerably heavier calves than did Brahman or Hereford cows when all three types of cows were bred to Hereford bulls. In a cooperative study, using King Ranch cattle, Black, Semple and Lush (1934) found that Brahman x Hereford and Brahman x Shorthorn calves were heavier at weaning and more profitable than purebred Hereford and Shorthorn cattle. Experiments with Brahman cattle in Louisiana by Bray (1933) resulted in the conclusion that it was advantageous to introduce some Brahman blood into commercial beef herds in the Gulf Coast Region. An analysis of 16 years of breeding studies at the Iberia Livestock Experiment Farm at Jeanerette, Louisiana, led Rhoad and Black (1943) to recommend that for the Gulf Coast Region, Brahman hybrid beef-type bulls be used on range cows with one-half to three-fourths the blood of a pure beef breed. They further recommended that one parent of the hybrid bulls should be of the same pure beef breed that sired the range cows and the other parent predominantly of Brahman breeding and of acceptable beef-type conformation. Baker and Black (1950) reported that Brahman x Angus calves averaged 57 lb. heavier at weaning than purebred Angus calves and that Africander x Angus calves also exceeded the purebred Angus calves in weaning weight.

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A study by McCormick and Southwell (1957) in Georgia, showed that Brahman x Hereford calves had significantly heavier birth weights than Angus x Hereford calves, and also somewhat heavier weaning weights. The Angus x Hereford calves, however, graded slightly higher. Kidder and Chapman (1952) reported on work in Florida in which progeny from Brahman x Angus, Brahman x Devon, and the reciprocal crosses were superior in birth weight and weight gains to the purebred lines of these crosses.

Crossbreeding studies in other sections of the country included work in Ohio by Gerlaugh, Kundle, and Rife (1951) which reported that in crossing Angus and Hereford cattle, crossbred calves from both the Hereford and the Angus cows outgained the corresponding groups of purebred calves from birth to weaning. Knapp, Baker, and Clark (1949) reported that Shorthorn x Hereford calves were heavier at weaning than purebred Herefords in an experiment conducted at Miles City, Montana.

The results from crossbreeding beef cattle have shown that, in general, increases in weaning weight can be expected when the different breeds are crossed. The most noteworthy increases have been realized in the Southern Region when Brahman cattle have been a part of the crossbreeding studies. The experiment to be reported in this paper was established in 1952 in order to compare several different breeds of beef cattle and several different crosses among these breeds under similar managerial and environmental conditions. The criteria of evaluation in this report are weaning weights and slaughter calf grades.

Experimental

The project was initiated by forming six herds each of 32 cows. These herds were composed of eight cows from the Angus, Hereford, Brahman, and Brangus breeds. The Brangus cows were secured from the Brahman-Angus line of cattle developed at the Iberia Livestock Experiment Farm at Jeanerette, Louisiana, and were treated as a pure breed of cattle. The term Brangus will be used throughout this paper to describe these cattle in order to distinguish them from the crosses made in this study between Brahman and Angus cattle. Some of the Brahman females were produced at this station while others were purchased from producers in Louisiana and Texas. The Angus and Hereford females were derived from similar sources, most of these cattle being purebred although some high grade cattle were used in each of these breeds. An effort was made to select females which would be as representative of the breeds as possible, with no attempt being made to choose outstanding animals in any case. The mature weights attained by the females of the Hereford, Brangus, Angus and Brahman breeds during the course of this experiment were 1210, 1195, 1155, and 1115 lb., respectively. The cows were weighed in the spring and fall of each year,

and the average mature weights were arrived at by selecting the heaviest weight recorded for each cow after reaching 4 years of age.

Each of the six herds thus formed was bred to a bull of a different breed. Bulls from Angus, Brahman, Brangus, Charolaise, Hereford and Shorthorn breeds were used. A new bull was selected each year to represent each of these breeds and the cow herds were rotated to a bull of a different breed annually. Thus, over the entire experimental period (1952–1957) each cow herd was mated to bulls of five different breeds. Bulls of the Angus, Brahman, Hereford and Shorthorn breeds were furnished from the purebred herds maintained by the University. Two of the Charolaise bulls were the property of the station, while the remaining Charolaise bulls were loaned to the station by the Turner Ranch, Weslaco, Texas. The Brangus bulls were supplied by the Iberia Livestock Experiment Farm, Jeanerette, Louisiana. Most of the bulls used in this study were 3 or 4 years old, although one Charolaise bull was only 18 months of age and a few 5- and 6-year-old bulls were used. The average weights of the Angus, Brahman, Brangus, Charolaise, Hereford and Shorthorn bulls were 1330, 1590, 1680, 1575, 1595, and 1555 lb., respectively. Differences in age and condition of the bulls do not allow these figures to be used as accurate measures of the mature weights of these bulls. However, the averages do point out that the Angus bulls were far lighter than the bulls of the other five breeds, a difference which is not due to either condition or age.

The six cow herds formed at the beginning of this experiment were maintained separately throughout the 5 years, replacements being made only when necessary through loss of cattle due to bloat, disease or reproductive failure. The females produced in this experiment have been retained and are being used in further breeding studies. The steers have been fed out and their performance will be reported in a separate paper.

The management practices were those generally recommended for production of beef cattle in the Gulf Coast Region. The breeding season was from April 1st to July 1st, pasture mating being practiced. Birth date, birth weight, and sex of each calf were recorded for each calf within 24 hours after birth. Weights were recorded at 28-day intervals thereafter. When the oldest calf approached 180 days, weights were taken at two-week intervals. Castration and dehorning were carried out early in April, toward the end of the calving season. The heifer calves were given a slaughter calf grade, a feeder calf grade, and a breeding grade at weaning time. The steer calves were given a slaughter calf grade and a feeder grade at that time. No calves on this experiment were creep-fed.

The calves were all weaned at the same time, averaging approximately 8 months of age. Weaning weights were standardized to 180 days of age by prorating the gains from the 14-day weights which included 180 days of age. The weaning weights were also adjusted for the age of dam.

Since no age of dam adjustment factors were available from this station, averages of the adjustment factors for each age group found at several different stations were used. These averages resulted in the addition of 54, 40, 22, 11, 0, 12, and 25 lb. to the weights of calves from 2-, 3-, 4-, 5-, 6 to 8-, 9-, and 10-year-old dams. The adjustment figure for 10-year-old dams was used for calves from older dams. The weights of the heifer calves were adjusted for sex by the addition of 30 lb. to their 180-day weights, the difference in sexes found in this study. Throughout the remainder of this paper the adjusted 180-day weights will be referred to as weaning weights. No adjustments were made on the data for slaughter calf grades.

The data were analyzed by the method of fitting constants as described by Kempthorne (1952) for a 2-way classification with unequal numbers and no interaction. The model was

$$y_{ijk}=\mu+a_i+b_j+e_{ijk}$$

where y_{ijk}=the observation on the kth animal for the jth breed group in the ith year

$i=1,\ldots,5$

$j=1,\ldots,24$

μ=an effect common to all animals

a_i=an effect common to all calves born in the ith year

b_j=an effect common to all calves born in the jth year

e_{ijk}=an effect common to each individual animal

The constants are the values of μ, a, and b in the model. The normal equations necessary to estimate the constants were determined and these equations solved by matrix inversion. The elements of this matrix inverse were used also to determine standard errors, and in tests of significance of differences among constants and groups of constants.

The method for determining standard errors of constants and combinations of constants estimated in the analysis of this experiment is described below.

Consider the following linear function of these constants:

$$\lambda = \hat{\mu} + f_1 + f_2 \qquad (1)$$

where $$f_1 = \frac{\hat{a}_1 + \hat{a}_2 + \ldots + \hat{a}_{n_1}}{n_1} = \frac{1}{n_1}\sum_{i=1}^{n_1} \hat{a}_i,$$

and $$f_2 = \frac{\hat{b}_1 + \hat{b}_2 + \ldots + \hat{b}_{n_2}}{n_2} = \frac{1}{n_2}\sum_{j=1}^{n_2} \hat{b}_j,$$

The variance of the linear function (1), assuming that $\sigma_e^2=1$, is

$$\sigma^2_{\lambda} = \sigma^2_{\hat{\mu}} + \sigma^2_{f_1} + \sigma^2_{f_2} + 2\sigma_{\hat{\mu}f_1} + 2\sigma_{\hat{\mu}f_2}$$

where $\sigma_{\mu f_1}$ and $\sigma_{\mu f_2}$ are covariance terms. Values for the terms in this equation are obtained from elements of the inverse matrix as follows:

$$\sigma^2_{\hat{\mu}} = c^{\mu\mu}$$

$$\sigma^2_{f_1} = \frac{1}{n_1^2}\left(c^{a_1a_1} + c^{a_2a_2} + \ldots + c^{a_{n_1}a_{n_1}} + 2c^{a_1a_2} + 2c^{a_1a_3} + 2c^{a_{n_1-1}a_{n_1}}\right)$$

$$\sigma_{\mu f_1} = \frac{1}{n_1}\left(c^{\mu a_1} + c^{\mu a_2} + \ldots + c^{\mu a_{n_1}}\right)$$

etc.

where the c's are elements of the inverse matrix with the superscripts indicating the row and column of each element, and the n's denoting the number of constants included.

All tests of significance in this paper were carried out by means of Duncan's "New Multiple Range Test" (1955) which was extended by Kramer (1957) to give tests for differences among means with unequal replications.

Results and Discussion

Year. A total of 597 calves was weaned from the cows on this project during the 5 years in which it was conducted. The percentage of weaned calves has been consistently lower than expected. This is explained partially by loss of cows from bloat, cancer eye, disease, or barrenness, and by calf mortality at birth and during the suckling period. However, the largest contributing factor has been a low level of fertility in some of the bulls used in this experiment. Bulls have produced as few as 6 and 8 calves out of a possible 32, in some cases. Most difficulty has been encountered with Brangus and Brahman bulls, with the Angus and Hereford bulls performing considerably better in this respect. The estimated means for weaning weights and slaughter calf grades are presented in table 1. The average weaning weights do not differ greatly, with the exception of 1957. The large drop in average weaning weight for this year is due primarily to the poor performance of the Shorthorn bull that sired these calves. The Shorthorn crosses in 1957 averaged 51 lb. lighter than the average of the 4 preceding years. This bull sired eight Shorthorn x Brangus calves whose average weaning weight was 85 lb. lighter and six Shorthorn x Brahman calves whose average weaning weight was 83 lb. lighter than the averages for the weights of these two crosses for the preceding 4 years. The Shorthorn x Angus and Shorthorn x Hereford crosses averaged 27 and 21 pounds lighter than the preceding 4 years. Had the 1957 Shorthorn calves attained the weight of the preceding 4 years, then the 1957 average weaning weight would have been very similar to that of the previous year.

TABLE 1. ANNUAL RESULTS FOR WEANING WEIGHTS AND SLAUGHTER CALF GRADES

Year	No. of calves	Weaning weights		Slaughter calf grades[a]	
		Estimated mean, lb.[b]	Standard error, lb.[c]	Estimated mean	Standard error[d]
1953	117	418.52	4.61	13.77	.173
1954	102	430.32	4.95	13.47	.186
1955	124	427.55	4.45	10.59	.167
1956	117	420.43	4.61	11.39	.173
1957	137	406.52	4.23	10.38	.159

[a] Grading system: 17–15, prime; 14–12, choice; 11–9, good; 8–6, commercial; 5–3, utility.
[b] Estimated by least squares.
[c] Error standard deviation for weaning weights=49.09 lb.
[d] Error standard deviation for slaughter calf grades=1.84.

The calves were graded by the same committee for each of the 5 years. The slaughter calf grades show more variation than would be expected from year to year.

Sire Groups. The data resulting from this experiment can be grouped in several different ways which yield meaningful information. Since all breeds of bulls were bred to the same four breeds of cows it is of interest to compare the weaning weights of the calves sired by the six different breeds of bulls. These data are presented in table 2 along with the standard errors and tests of significance among the different sire groups. The Charolaise bulls have consistently sired calves with heavier weaning weight than have the other breeds of bulls. However, there is no significant difference between the Charolaise and Hereford bulls although the Charolaise bulls show a highly significant difference from all other breeds. The table shows that the Hereford bulls have also performed very well, siring calves that were considerably heavier than those of the four breeds of bulls ranked below them. The Shorthorn, Brahman, and Brangus bulls performed quite similarly with respect to weaning weights. The Angus bulls consistently yielded calves whose weaning weights were considerably below average.

TABLE 2. WEANING WEIGHTS RANKED BY SIRE GROUPS AND TESTS OF SIGNIFICANCE AMONG SIRE GROUPS

Breed of sire	No. of calves	Estimated mean, lb.	Standard error, lb.	Difference lb.				
				Hereford	Shorthorn	Brahman	Brangus	Angus
Charolaise	101	448.13	4.92	11.49	31.14**	31.94**	34.90**	55.30**
Hereford	113	436.64	4.63		19.65**	20.45**	23.41**	43.81**
Shorthorn	99	416.99	5.01			0.80	3.76	24.16**
Brahman	88	416.19	5.35				2.96	23.36**
Brangus	86	413.23	5.41					20.40**
Angus	110	392.83	4.70					

**Significant at the 1% level.

TABLE 3. SLAUGHTER CALF GRADES RANKED BY SIRE GROUPS AND TESTS OF SIGNIFICANCE AMONG SIRE GROUPS

Breed of sire	No. of calves	Estimated mean	Standard error	Differences				
				Brahman	Shorthorn	Angus	Charolaise	Brangus
Hereford	113	12.86	0.174	0.76**	0.79**	1.16**	1.18**	1.75**
Brahman	88	12.10	.201		.03	.40	.42	.99**
Shorthorn	99	12.07	.188			.37	.39	.96**
Angus	110	11.70	.176				.02	.59*
Charolaise	101	11.68	.184					.57*
Brangus	86	11.11	.203					

*Significant at the 5% level.
**Significant at the 1% level.

A comparison of the performance of the different breeds of bulls with respect to slaughter calf grades presented in table 3 shows some noteworthy changes from the results for weaning weights. The calves sired by the Charolaise bulls, although demonstrating heavier weaning weights, rank fifth in the slaughter calf grade comparison. These calves have an excellent rate of growth but received a lower grade because of lack of finish. The calves sired by Hereford bulls have been graded high consistently, and the grades of these calves show a superiority over the other calves that is highly significant. The Brangus bulls sired calves whose grades were considerably below those of all other breeds of bulls.

Dam Groups. Since the four breeds of cows used in this experiment were all bred to the six different breeds of bulls, it is of interest to compare the performance of the cows. The data are grouped by breed of dam in table 4, together with tests of significance among the four breeds of cows. These results have been very consistent throughout the 5 years of this experiment, and have shown the Brangus cows to raise calves that were well above the average of those raised by the other three breeds of cows. The Brahman cows also demonstrated their ability to raise a calf to a heavy weaning weight under the conditions prevailing in this region. The Angus and Hereford cows raised calves whose weaning weights were quite similar and markedly lighter than those of the Brangus and Brahman cows. The results demonstrate clearly the superior mothering ability of

TABLE 4. WEANING WEIGHTS RANKED BY DAM GROUPS AND TESTS OF SIGNIFICANCE AMONG DAM GROUPS

Breed of dam	No. of calves	Estimated mean, lb.	Standard error, lb.	Differences, lb.		
				Brahman	Angus	Hereford
Brangus	163	442.74	3.87	17.75**	31.94**	38.59**
Brahman	150	424.99	4.05		14.19*	20.84**
Angus	145	410.80	4.19			6.65
Hereford	139	404.15	4.19			

* Significant at the 5% level.
** Significant at the 1% level.

females possessing some or all Brahman breeding, in this region of the country.

An inspection of table 5, presenting the results of slaughter calf grades by breed of dam, shows a pattern quite similar to that of weaning weights Although the differences are relatively smaller, the ranking remains the same with Brangus and Brahman cows raising calves with higher slaughter calf grades.

Breed Groups. The performance with respect to weaning weights, of the 24 different types of cattle produced in this work is presented in table 6. It can be seen that combinations of those bulls siring the heavier calves, and those cows raising the heavier calves, rank among the top groups. The vital role of the dam is emphasized when it is seen that 8 of the first 10 crosses involve either Brangus or Brahman females. In fact, 5 out of

TABLE 5. SLAUGHTER CALF GRADES RANKED BY DAM GROUPS AND TESTS OF SIGNIFICANCE AMONG DAM GROUPS

Breed of dam	No. of calves	Estimated mean	Standard error	Differences		
				Brahman	Angus	Hereford
Brangus	163	12.23	0.145	0.19	0.30	0.75**
Brahman	150	12.04	.152		.11	.56*
Angus	145	11.93	.157			.45*
Hereford	139	11.48	.157			

* Significant at the 5% level.
** Significant at the 1% level.

the 6 matings with Brangus cows are included among the first 10 groups. The distinct advantage of having Brahman breeding on the female side is clearly demonstrated by these results. The Brahman bulls have also exhibited the fact that they can be used to advantage, when it is noted that when they are bred to Brangus, Angus, or Hereford cows there is an increase in weaning weight over the straight bred animals. This effect is particularly noteworthy in the Brahman x Angus cross.

While the Brangus cows have proved to be outstanding dams, the Brangus bulls have been disappointing in their performance. Calves sired by the Brangus bulls have had low weaning weights and also low slaughter calf grades. The Hereford cattle have performed in the opposite fashion, with the bulls siring heavy calves with good slaughter calf grades and the cows raising lighter calves with poorer slaughter calf grades. Although the Angus cows raised calves with slightly heavier weaning weights and slightly better slaughter calf grades than did the Hereford cows, the Angus bulls have consistently sired the lightest calves. This appears to be due in large part to the smaller size of the Angus bulls. The importance of the size of bulls has been pointed out previously by Gerlaugh *et al.* (1951).

The data show clearly, that under the conditions of this experiment,

TABLE 6. WEANING WEIGHTS AND SLAUGHTER CALF GRADES RANKED BY BREED GROUPS AND TESTS OF SIGNIFICANCE AMONG BREED GROUPS

Weaning weights					Slaughter calf grades				
Breed group[a]	No. of calves	Estimated mean, lb.	Standard error, lb.	Tests of Significance[b]	Breed group[a]	No. of calves	Estimated mean	Standard error	Tests of Significance[b]
C x B	24	466.86	10.07		H x BA	30	13.47	.337	
C x BA	29	466.46	9.13		H x B	29	12.82	.342	
H x BA	30	465.03	8.97		S x B	23	12.78	.385	
H x B	29	455.79	9.13		B x A	16	12.71	.461	
S x B	23	449.64	10.27		H x H	27	12.65	.355	
C x A	25	440.85	9.82		B x BA	28	12.61	.348	
B x BA	28	438.52	9.28		H x A	27	12.50	.355	
S x BA	24	436.32	10.07		C x B	24	12.49	.377	
BA x BA	24	433.45	10.07		S x BA	24	12.19	.377	
B x A	16	431.27	12.28		A x BA	28	12.07	.348	
B x H	21	420.07	10.75		A x B	29	11.93	.342	
C x H	23	418.35	10.27		B x H	21	11.85	.403	
A x BA	28	416.64	9.28		A x A	27	11.83	.355	
H x H	27	415.06	9.46		S x A	31	11.83	.331	
BA x H	21	411.78	10.75		C x A	25	11.66	.368	
H x A	27	410.67	9.46		C x BA	29	11.57	.342	
BA x A	19	406.89	11.35		S x H	21	11.50	.403	
A x B	29	401.95	9.13		BA x BA	24	11.49	.377	
BA x B	22	400.82	10.51		B x B	23	11.26	.385	
S x H	21	391.68	10.75		BA x A	19	11.05	.425	
S x A	31	390.33	8.83		C x H	23	10.99	.385	
A x A	27	384.79	9.46		BA x B	22	10.98	.394	
B x B	23	374.89	10.27		A x H	26	10.98	.362	
A x H	26	367.94	9.64		BA x H	21	10.91	.403	

[a] The abbreviations used in this table are as follows: A=Angus; B=Brahman; BA=Brangus; C=Charolaise; H=Hereford; S=Shorthorn. The sire is presented first.

[b] The tests of significance are expressed so that any two means included by the same line are not significantly different, and any two means not included by the same straight line are significantly different. The dotted line indicates that the mean for that group differs significantly from the uppermost mean in that line, although the means below the dotted line do not differ significantly ($P<.05$).

English breeds have not crossed to advantage. Reciprocal crosses between the Angus and Hereford breeds, and crosses of Shorthorn bulls on Angus and Hereford cows have yielded very poor results. It can also be seen that crosses involving Brahman and English breeding have given excelient results.

It was impossible to include Charolaise females in this study, but the crossing of Charolaise bulls on four different breeds of cows has yielded considerable information on the potentialities of this breed. These bulls have performed in a fashion very similar to the bulls of the English breeds in that considerably heavier weaning weights are attained when they have been mated to Brahman and Brangus cows than when mated to Angus and Hereford cows. However, even when crossed with females of English breeding, the Charolaise bulls have demonstrated their ability to sire heavy weaning calves.

It has been pointed out previously that the Shorthorn bull used in the last year of this experiment, sired an exceptionally light group of calves, particularly in the Shorthorn x Brangus and Shorthorn x Brahman crosses. These weights were so different from those of previous years that it is felt that this point should be emphasized and that better results might be expected from these crosses than would be anticipated from the figures presented in table 6.

An examination of the slaughter calf grades received by the different breed groups, presented in table 6, shows that 7 of the 10 top ranked crosses were also among the 10 top ranking crosses for weaning weights. Since a large proportion of the calves produced in the Gulf Coast Region are sold as slaughter calves, it is of importance to produce calves that will grade high in addition to having heavy weaning weights. The Hereford and Shorthorn bulls have shown their ability to sire such calves when bred to Brangus and Brahman females. It is noticeable again that most of the crosses receiving high slaughter calf grades were raised by Brangus and Brahman females. Although the Charolaise bulls sired calves with heavy weaning weight, table 6 shows that they do not attain a correspondingly high slaughter calf grade. These calves have excellent size and scale but do not have the same degree of finish that is generally associated with the English beef breeds. The English beef breeds show to better advantage with respect to slaughter calf grade than they do with respect to weaning weight. All breed groups containing solely English breeding rank noticeably higher in this trait, than when ranked for weaning weights.

Summary and Conclusions

A study was initiated in 1952 to evaluate the performance of different breeds of beef cattle, and crosses among these breeds, in the Gulf Coast region. An analysis of the results of 5 years of work with respect to weaning weights and slaughter calf grades has yielded considerable infor-

mation as to the merits of 24 different types of cattle, particularly in the Gulf Coast and similar regions. It has been found that Brangus and Brahman females raise calves that are not only heavier at weaning but also grade higher. The heaviest weaning weights are generally reached with the use of Charolaise, Hereford, and Shorthorn bulls on the Brangus and Brahman cows. Although Charolaise bulls have sired the heaviest calves, the slaughter calf grades of these calves are lowered because of their lack of finish. Although no Charolaise females were used in this study, the Charolaise bulls have demonstrated their ability to cross well with all four breeds of cows used in this study. Crosses among Brahman and other breeds of cattle have shown a considerable advantage over the pure breeds with respect to weaning weights although the advantage is not so marked when slaughter calf grades are considered. These crosses are generally superior when the Brahman breeding is in the females. The data indicate that little or no advantage is to be gained by crossing the English beef breeds under the conditions of this experiment.

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PORK QUALITY. I. INFLUENCES OF SOME FACTORS ON PORK MUSCLE CHARACTERISTICS

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CONSUMER preference studies have shown that both quantity and quality characteristics are important to the consumer of pork. Birmingham *et al.* (1953, 1957) reported that laboratory and family taste panels preferred pork from U. S. Medium grade hogs over pork from other grades. Grade did not influence processing yields. Other work, however, has indicated that carcass fatness may contribute positively to the palatability and processing yields of pork (Saffle and Bratzler, 1957). Self *et al.* (1957) studied the association of carcass grade and weight with the composition and quality of lean cuts. Desirable hams and loins were observed more frequently in the U. S. No. 1 grade carcasses. Loins from U. S. No. 2 and No. 3 grade carcasses were found to have more marbling and seam fat than loins from U. S. No. 1 or Medium grade carcasses. Soft, watery loins were more frequent in the U. S. Medium grade carcasses than in any other group. Pale, watery, "degenerated" pork muscle has been a concern of Ludvigsen (1954). This altered muscle structure was observed more frequently in the summer.

This investigation was undertaken to establish the influences exerted by season, sex, breed, age, degree of fatness, and degree of muscling on some of the characteristics of fresh pork muscle regarded as important in processing and to palatability. Subsequent investigations will be reported pertaining to the relative importance of these muscle characteristics to the processor and consumer of pork.

Materials and Methods

The general plan of the experiment included utilization of 321 hogs entered in the Improvement Program of the Ohio Swine Improvement Association.[2] All hogs were housed in the Swine Evaluation Station and were treated uniformly throughout the growing-fattening period.[3] Animals were taken off feed on the evening prior to the day of slaughter. Subsequent procedures mentioned include only those pertaining to this experiment.

Slaughter, Measurement, and Cutting Procedures. Standard slaughter and cutting methods were used as described in the Report of Proceedings

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[2] Outlines of the objectives and policies of the Ohio Swine Improvement Association are available through the Department of Animal Science, Ohio State University.

[3] Rations fed were reported by Bruner *et al.* (1958).

of the Fifth Annual Reciprocal Meat Conference. Carcasses were chilled for approximately 48 hours at 32–34° F. after which time they were measured and cut. Backfat thickness as reported here represents an average of the backfat measurements at the first rib, last rib, and the last lumbar vertebra. The cross sectional area of the *longissimus dorsi* muscle was determined by the method described by Cahill *et al.* (1953). "Percent lean cuts" and "percent fat for lard" are based on the weight of the chilled carcass.

TABLE 1. FREQUENCIES OF MUSCLE COLORS

	Light		Greyish-Pink		Dark		Two-toned	
Items	N	%	N	%	N	%	N	%
Season**								
Spring[a]	60	38.7	75	48.4	12	7.7	8	5.2
Fall[b]	41	24.7	102	61.4	20	12.0	3	1.8
Sex								
Barrows	56	35.9	79	50.6	14	9.0	7	4.5
Gilts	45	27.3	98	59.4	18	10.9	4	2.4
Breed[c]**								
Poland China	24	60.0	13	32.5	1	2.5	2	5.0
Landrace	13	48.1	11	40.7	1	3.7	2	7.4
Hampshire	20	41.7	26	54.2	2	4.2	0	0.0
Spt. Poland C.	17	41.5	22	53.7	2	4.9	0	0.0
Berkshire	6	20.7	18	62.1	4	13.8	1	3.4
Yorkshire	18	16.4	72	65.5	16	14.5	4	3.6

[a] December 1 to June 1 farrow.
[b] June 1 to December 1 farrow.
[c] Only those breeds represented by 20 or more individuals are listed.
** $P<.01$.

Muscle Classification. Visual appraisal was made of the cross sectional surface of the *longissimus dorsi* muscle at the tenth rib. Observations were made immediately after cutting of the carcasses when internal temperatures were approximately 36° F. Loins were classified subjectively on the bases of muscle color, intramuscular fat or marbling, and muscle firmness. Greyish-pink color was regarded as a standard. Four color categories were then established. Pronounced deviations from greyish-pink color were recorded as "light" or "dark". Loins that lacked one predominant uniform color were termed "two-toned." The amount of intramuscular fat seen at the tenth rib section was evaluated in descriptive terms as "slight", "moderate", or "abundant". The firmness of the loins was described as "soft", "intermediate", or "firm". This factor was measured by a combination of touch and visual appraisal.

The data were statistically analyzed as outlined by Ostle (1956). Chi-square tests for independence of samples were performed on the season, sex,

and breed data. Correlation coefficients were calculated between muscle types and age, measures of fatness, and measures of muscling. Adaptation of muscle classification data to correlation analyses required the numerical coding of the various muscle types.

Results and Discussion

Season. Data for this experiment were collected from hogs raised during different periods of the year. Pigs farrowed between December 1 and June 1 were slaughtered during the warm summer weather and are referred to as Spring pigs. Colder weather conditions prevailed when Fall (June 1 to December 1 farrow) pigs were slaughtered.

TABLE 2. FREQUENCIES OF DEGREES OF MARBLING

	Slight		Moderate		Abundant	
Items	N	%	N	%	N	%
Season**						
Spring[a]	90	58.0	46	29.7	19	12.3
Fall[b]	56	33.7	76	45.8	34	20.5
Sex						
Barrows	64	41.0	59	37.8	33	21.2
Gilts	82	49.7	63	38.2	20	12.1
Breed[c]**						
Landrace	18	66.7	7	25.9	2	7.4
Poland China	26	65.0	8	20.0	6	15.0
Yorkshire	60	54.5	41	37.3	9	8.2
Hampshire	24	50.0	22	45.8	2	4.2
Spt. Poland C.	10	24.4	19	46.2	12	29.3
Berkshire	4	13.8	13	44.8	12	41.4

[a] December 1 to June 1 farrow.
[b] June 1 to December 1 farrow.
[c] Only those breeds represented by 20 or more individuals are listed.
** $P<.01$.

Tables 1, 2, and 3 illustrate numerically the influence of season on the frequency of muscle types. Pigs grown and fattened during cool weather yielded darker, more highly marbled loin eye muscle ($P<.01$). Firmness was unaffected by season.

Sex. Differences in the color and firmness of the *longissimus dorsi* muscle, as influenced by the sex of the animal, were insignificant. Difference in marbling approached significance ($P<.05$) with barrow loins exhibiting more intramuscular fat than loins from gilts. Frequencies and percentages are listed in tables 1, 2, and 3.

Breed. Breed differences were highly significant. Tables 1, 2, and 3 indicate that certain of the breeds tended to produce loin muscles of a light colored, slightly marbled, soft nature, while other breeds produced dark, abundantly marbled, firm loins at comparatively high rates. The breeds

TABLE 3. FREQUENCIES OF DEGREES OF FIRMNESS

Items	Soft		Intermediate		Firm	
	N	%	N	%	N	%
Season						
Spring[a]	74	47.7	36	23.2	45	29.0
Fall[b]	63	38.0	45	27.1	58	34.9
Sex						
Barrows	61	39.1	39	25.0	56	35.9
Gilts	76	46.1	42	25.5	47	28.4
Breed[c]**						
Landrace	17	63.0	5	18.5	5	18.5
Poland China	25	62.5	10	25.0	5	12.5
Hampshire	29	60.4	9	18.8	10	20.8
Spt. Poland C.	16	39.0	12	29.3	13	31.7
Yorkshire	37	33.6	33	30.0	40	36.4
Berkshire	9	31.0	6	20.7	14	48.3

[a] December 1 to June 1 farrow.
[b] June 1 to December 1 farrow.
[c] Only those breeds represented by 20 or more individuals are listed.
** $P<.01$.

are ranked on the basis of the percentages calculated in the various muscle groups. The limited usefulness of the Chi-square test on data of this type has prevented comparison between breeds. In addition, the differences between breeds prohibits the comparison of individual breeds to the average of all breeds. The data reported here may serve merely as an indication of the influences of heriditary factors.

Age. Correlation coefficients (table 4) suggest that the age of the animal was not important to color, marbling, or firmness. Even though the range of ages of the hogs used was very narrow, it could be concluded that among butcher hogs whose ages are normally within a narrow range, little of the variation in muscle types could be accounted for by variation in age.

Carcass Meatiness and Fatness. Significant and highly significant correlation coefficients were calculated for the degrees of marbling and firmness with various measures of meatiness and fatness (table 4). These statistics show that loins from meaty carcasses were slightly less firm and had less marbling than loins from fatter carcasses. The correlations are

TABLE 4. CORRELATION COEFFICIENTS OF MUSCLE CHARACTERSTICS, MEASURES OF MEATINESS, MEASURES OF FATNESS AND AGE

	Marbling	Firmness	Loin eye size	% lean cuts	% fat for lard	Average backfat	Age
Color	0.26**	0.57**	0.01	0.01	0.01	0.05	0.05
Marbling		.51**	—.03	—.18**	.14**	.06	—.05
Firmness			—.09	—.17**	.14**	.13*	.03

* $P<.05$.
** $P<.01$.

low, however, and account for only a small portion of the variation. Color was not related to these carcass data.

Interrelationships of Muscle Characteristics. Visual examination of the loins permitted observation of certain relationships existing between muscle characteristics. Color, degree of marbling, and degree of firmness were all positively associated with each other ($P<.01$; table 4). The firmness of chilled intramuscular fat may have been responsible for the relationship between marbling and firmness; however, additional data is needed to justify this conclusion. The association of muscle color with the degree of firmness is best explained as concurrent results of hydration. As suggested by Hamm (1953), light penetrates deeply into firm, hydrated product causing the color to appear dark. The low but highly significant coefficient calculated between color and degree of marbling is more difficult to explain. Subsequent research is needed to establish this relationship.

Summary

Three hundred twenty-one fresh pork loins were observed and classified on the bases of the color, marbling, and firmness of the *longissimus dorsi* muscle. Carcasses from hogs raised and slaughtered during the cool weather season were found to have darker, more highly marbled loins than those from hogs raised in warmer weather. The breed of the animal was highly significant in its influence on muscle types. Meaty carcasses with minimum amounts of fat were found to yield soft, slightly marbled loin muscles at a slightly higher rate than the less meaty, fatter carcasses. The correlations were low, however, and explain little of the variation in muscle types.

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REPRODUCTION IN THE YEARLING EWE AS AFFECTED BY BREED AND SEQUENCE OF FEEDING LEVELS. I. EFFECTS ON OVULATION RATE AND EMBRYO SURVIVAL [1]

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THE practice of "flushing" ewes by improvement of their nutrition for a short time just prior to breeding is often recommended as a method of increasing the lamb crop. Clark (1934) indicated an increase in ovulation rate of mature ewes due to "flushing" for a period as short as approximately 3 weeks in one of two trials. McKenzie and Terrill (1937) and El-Shiekh *et al.* (1955) have demonstrated consistent increases in ovulation rates from higher levels of feeding over an extended period of time.

The high level of feed which was found by El-Shiekh *et al.* (1955) to increase ovulation rates was shown, also, to increase embryo mortality when measured at 40 days of gestation.

McKenzie and Terrill (1937), Clark (1934) and others have reported breed differences in ovulation rate with the English breeds producing a greater number of ova than the fine wool breeds.

This study was made to test the effects of two levels of feeding on ovulation rate and embryo survival in different breeding groups of yearling ewes. The feed levels were given in different sequences and for different periods of time.

Materials and Methods

A total of 449 ewes was included in this study over a period of 3 years (1955–1957). All animals were purchased as feeder ewe lambs through a terminal market.

In March, 1955, 128 ewe lambs of mixed breeding (mainly Down and Corriedale) were divided at random into four sequences-of-feeding groups, one-half of each group being killed at 3 days and one-half at 40 days of gestation. Sixteen 2-year-old rams of similarly mixed breeding were used, each being mated to one ewe within each feed level of the 3-day and 40-day gestation groups. The total experimental time was divided into three feeding periods as follows: (1) Growing period (March 11 to October 1), (2) prebreeding or flushing period (October 1 to breeding for each individual ewe, beginning October 15) and (3) portion of the gestation period (3 or 40 days).

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In January, 1956, 156 ewe lambs and in April, 1957, 165 ewe lambs were placed on experiment. Each year approximately one-half (84 and 81, respectively) were predominantly Hampshire breeding and the other one-half (72 and 84) predominantly Columbia breeding. Both of these latter groups were considered grade Columbias although there probably was an appreciable mixture of Rambouillet breeding in 1957. One-half of each breeding group of ewes was bred to four purebred Hampshire and one-half to four purebred Columbia rams resulting in four different breeding groups (Straight-bred Hampshire, Straight-bred Columbia, and their reciprocal crosses). To insure better sampling of the breeds, rams unrelated to each other for at least three generations were used. Different rams were used each year. The ewes within each of these breeding groups were assigned at random into six different sequences of feeding levels; one-third were killed at 25 days and two-thirds at 140 days of gestation. The total experimental time was divided into four feeding periods with the first two being similar to those in 1955. The experiment began January 19 in 1956 and April 4 in 1957; the prebreeding or flushing period was started on September 23 and breeding on October 7 in both years. The third period was from breeding to 25 days of gestation and the fourth from 25 to 140 days of gestation.

In all years the feeding groups were composed of different sequences of hay only (limited feed, L) and hay plus grain (full feed, F) rations. The hay-only ration consisted of alfalfa-grass hay fed *ad libitum*. The hay-plus-grain ration included a full feeding of a grain mixture [3] (approximately 2.0 lb./head/day) in addition to hay *ad libitum*. Ewes in the hay plus grain group were estimated to have received approximately 20% more TDN than those in the hay-only group, the minimum requirement for specific nutrients being met by each of these rations according to NRC recommendations. The calculated nutritive ratios were similar for the two levels of feeding (1:5.5). These are only rough approximations, however, because the amount of feed actually consumed by each group was not measured.

All ewes were vaccinated for overeating disease and drenched with phenothiazine and lead arsenate for internal parasites before being put on experiment. Subsequent drenchings were given as need was indicated.

The ewes were placed on pasture for approximately three weeks during August and September, 1955. The animals were kept in drylot throughout the experimental period, in 1956 and 1957, and only hay of U.S. No. 1 grade, containing at least 50% alfalfa, was fed during 1957. This was done to minimize fluctuations in the quality of roughage which might be a possible complication in measuring some of the feed effects. Tables

[3] Grain Mixture: Cracked shelled corn, 500; whole oats, 300; linseed meal, 100; and wheat bran, 100.

After June 2, 1956, the amounts of cracked shelled corn and whole oats were changed to 300 parts and 500 parts, respectively. Trace mineralized salt and bonemeal were available free choice throughout the experiment.

TABLE 1. DESIGN OF EXPERIMENT AND THE NUMBER OF ANIMALS STARTED IN EACH EXPERIMENTAL GROUP (1955)

Experimental periods			Stage of gestation at slaughter	No. of ewes
1[a]	2[b]	3[c]		
L	L	L	3	16
L	L	L	40	16
L	F	F	3	16
L	F	L	40	16
F	F	F	3	16
F	F	L	40	16
F	F	F	3	16
F	F	F	40	16

L=Hay only. F=Hay plus a full feed of grain.
[a] Level of feeding from beginning of experiment to October 1, approximately 7 months.
[b] Level of feeding from October 1 to breeding beginning October 15.
[c] Level of feed from breeding to 3 or 40 days of gestation.

1 and 2 show sequence of feeding for each of the experimental groups, the number of animals started on experiment, and the stage of gestation at which they were to be slaughtered for the different years.

Those ewes which received grain during the second experimental feeding period comprised the flushed groups (flushing in this study is used to denote short time grain feeding just prior to breeding). Ewes in these groups received grain for a minimum of 15 days (September 23–October 7). The first 12 days were used to get the animals on a full feeding of grain (1.5

TABLE 2. DESIGN OF EXPERIMENT AND THE NUMBER OF ANIMALS STARTED IN EACH EXPERIMENTAL GROUP (1956 AND 1957)

Experimental periods				Stage of gestation at slaughter	Hampshire sire				Columbia sire			
1[a]	2[b]	3[c]	4[d]		Hamp. dam		Col. dam		Hamp. dam		Col. dam	
					[e]	[f]						
L	L	L	—	25	4	3	4	3	4	4	4	3
L	L	L	L	140	5	3	4	4	5	3	4	3
L	L	L	F	140	5	3	4	4	5	4	4	4
L	F	L	—	25	4	3	4	3	4	4	4	3
L	F	L	L	140	5	4	4	4	5	3	4	3
L	F	L	F	140	5	4	4	4	5	3	4	4
F	F	F	—	25	4	7	4	7	4	7	4	7
F	F	F	L	140	5	7	4	7	5	7	4	7
F	F	F	F	140	5	6	4	7	5	6	4	7

L=Hay only. F=Hay plus a full feed of grain.
[a] Level of feeding from beginning of experiment to September 23.
[b] Level of feeding from September 23 to breeding beginning October 7 and continuing as ewes came in heat; average approximately 3 weeks.
[c] Level of feeding from breeding to 25 days of gestation.
[d] Level of feeding from 25 to 140 days of gestation.
[e] [f] No. of ewes started on experiment per sub-group for 1956 and 1957, respectively.

lb./head/day—on the basis of body weight, this is approximately equal to two lb./head/day for ewes grown out on full feed or from 1.4–1.5% of body weight in either case). They were then maintained at this level for the next 3 days after which they were bred as they showed heat.

The experimental rams were kept in a thrifty condition with a minimum of fatness. Their fertility was checked by gross examination of semen and by breeding and slaughtering ewe mates to get fertilization rate. All rams used showed high fertility.

Heat was determined by use of "marked" vasectomized rams. Checks were made twice daily (at approximately 12-hour intervals). The ewes were hand mated approximately 12 hours after first being found in heat. The matings were observed and checked grossly for vaginal insemination in 1955. In 1956 and 1957 vaginal samples were taken with a pipette and examined under the microscope for sperm. Animals refusing service to experimental rams after accepting a teaser ram were considered as not in heat and no further attempt was made to breed them at that time.

In 1955, ewes which returned to heat prior to 40 days in the 40-day group were rebred and slaughtered at three days (as replacements for 3-day ewes in which all of the eggs had not been recovered). This was done within the limits set by the feeding groups. In all cases these ewes furnished data on embryo survival.

In 1956 and 1957 most of the ewes returning to heat before 25 days post-breeding were slaughtered on the day of heat. Exceptions to this plan were those ewes assigned to be slaughtered at 140 days of gestation which had been on continuous, full or limited feeding from the beginning of the experiment. They were bred a maximum of three times to furnish more information for a fetal growth study (Foote, *et al.*, 1959). Although the corpora lutea at slaughter in these cases were produced from different cycles than those at the initial breeding, a maximum period of approximately one month was involved and changes in ovulation rate (corpora lutea count) would most likely be due to random variation rather than season or feed.

One ewe in 1956 and one in the early part of the study in 1957 showed heat after breeding and were found to be pregnant at slaughter. Because of an observed low fetal death rate after 25 days of gestation and to avoid loss of animals, ewes returning to heat after 25 days of pregnancy were not slaughtered during the rest of the study. This resulted in loss of information on ovulation rate in those ewes (four in all) actually losing their pregnancy during this period.

During the first week of September in 1957, those ewes receiving grain (continuous full-fed group only) accidentally received grain treated for insecticidal purposes with carbon tetrachloride and ethylene dichloride. This resulted in the death of two ewes and definite signs of sickness in at least seven more. All animals were thrown off feed. Their return to full feed, however, was quite rapid (2–3 weeks). There were no apparent

effects on subsequent growth rates as judged by comparisons with the previous year.

Data collected for this study included body weight at beginning of preflushing period (second feeding period), at breeding and at slaughter. The reproductive tract was examined for physical barriers to fertilization and the number of corpora lutea recorded. The tract was then dissected and flushed with physiological saline to recover ova in the 3-day ewes. The ova were checked for fertilization and abnormalities. At the later stages of gestation the fetuses were examined grossly for abnormalities. The carcass weight and a finish grade (ranging from 0 to 10 depending on degree of fatness) were taken on the 3-day animals in 1955. Preliminary analysis indicated that little embryonic death occurred after 25 days gestation. For this reason the 25- and 140-day data were combined in studying embryo survival.

Analysis of variance was by the method of unweighted means (Snedecor, 1956). Combining the results of the different years was done by pooling the individual probabilities (Fisher, 1944). Such probability pools were made in those cases where it appeared likely that the pooled probability would be less than 0.10.

Simple correlations calculated from pooled sums of squares and cross-products for each breed-feed group each year were used in the standard partial regression analysis. A test for heterogeneity of regression was made using the sub-group sums of squares and cross-products before they were pooled. The standard errors of the standard partial regressions, calculated as shown by Brandt (1928) and Wright (1934), were used in determining their significance. For purposes of analysis and interpretation of data the main variables—level of feed, breed of sire, breed of dam, and breed of maternal environment were considered as fixed.

Results and Discussion

Ovulation Rate. The ewes that were fed grain in addition to hay for 6 to 8 months before breeding produced a greater percentage of multiple ovulations than those receiving a ration of hay only. This was true for each year of the experiment and for each breed group studied. There were significant differences of 28% for ewes of mixed breeding in 1955, 48 and 32% in 1956 and 39 and 33% in 1957 for ewes of Hampshire and Columbia breeding, respectively (table 3). Flushing (grain feeding for approximately three weeks), as compared to the controls limited to a ration of hay, increased the percentage of multiple ovulations significantly in 1957 only. This was an increase of 20% for the Hampshire and 14% for the Columbia ewes. This can be compared to —3% and 0% for the same breeds, respectively, in 1956 and 11% for ewes of mixed breeding in 1955. There is some indication that Hampshire ewes may produce a larger number of ova than Columbia (table 3). The differences approached significance in

TABLE 3. EFFECT OF LEVEL OF FEEDING ON OVULATION RATE IN YEARLING EWES

Year	Breed	Total no. ewes	Hay plus grain (FF)			Hay, flushed with grain (LF)			Hay only (LL)		
			No. ewes	Multiple ovulations		No. ewes	Multiple ovulations		No. ewes	Multiple ovulations	
				No.	%		No.	%		No.	%
1955	Mixed	117	61	28	46	28	8	29	28	5	18
1956	Hampshire	86	28	22	79	29	8	28	29	9	31
	Columbia	71	23	14	61	24	7	29	24	7	29
1957	Hampshire	79	39	17	44	20	5	25	20	1	5
	Columbia	81	42	14	33	21	3	14	18	0	0

Test of significance: Feed, $P<0.01$ (for each year). LF *vs.* LL, $P<0.05$ (for 1957 only). Breed of dam, $P\cong0.08$ (1957); pooled $P=0.14$ (1956 and 1957).

1957 ($P=0.08$). A pooled probability for the two years was 0.14. In each year the response to an increase in level of feeding was greater, though not siginficantly, for the Hampshire than the Columbia.

The consistent increase in ovulation rate resulting from an increase in level of feed for an extended period of time during the growing period is in agreement with the work of McKenzie and Terrill (1937) and El-Shiekh *et al.* (1955). This period, however, is much longer than the flushing period of a few weeks. Those ewes grown out on grain were heavier than those in the flushed and limited fed groups (table 4).

Data were available on length of carcass (measured from anterior margin of first rib to aitch bone) at 25 days gestation for some of the animals in 1957. Comparisons with hay only showed that full feeding increased skeletal growth in both the Hampshire (66.8 *vs.* 63.4 cm.) and Columbia ewes (66.8 *vs.* 65.5 cm.); there was no apparent difference in the response of these two genetic groups to full feeding. In full-fed ewes ovulation rate may have been greater because of the fatness of the animal or because of a

TABLE 4. AVERAGE PREFLUSH WEIGHT[a] AND AVERAGE DAILY GAIN DURING THE FLUSHING PERIOD[b] FOR ANIMALS ON THE DIFFERENT LEVELS OF FEED

Year	Breed	Total no.	Hay plus grain			Hay, flushed with grain			Hay only		
			No. ewes	Preflush weight, lb.	Av. daily gain, lb.	No. ewes	Preflush weight, lb.	Av. daily gain, lb.	No. ewes	Preflush weight, lb.	Av. daily gain, lb.
1955	Mixed	117	61	137	−.01	28	101	0.16	28	102	0.08
1956	Hampshire	86	28	150	.13	29	104	.25	29	108	.07
	Columbia	71	23	142	.04	24	101	.32	24	100	.04
1957	Hampshire	79	39	133	.33	20	105	.27	20	95	.18
	Columbia	81	42	128	.25	21	97	.17	18	102	.10

[a] Average body weight for each feed group taken at the time the group to be flushed was started on grain.

[b] Average daily gain for the period of time from beginning of grain feeding, for those ewes being flushed, until they were bred and for comparable periods for the other feed groups. Period averaged approximately three weeks.

higher immediate nutritive state. It might also be ascribed to greater sexual development resulting from an acceleration in body development. The mechanism involved, however, in those cases in which flushing increased ovulation rate would be more logically attributable to changes in the immediate nutritive state of the animal.

An attempt was made to identify factors working within the sub-groups of breed, feed and year, which might have caused individual variation in multiple ovulation. Body weight, taken on all animals at the time the flushed group was started on grain (a preflush weight), was used as a measure of the way the animal had grown. The average daily gain for the period of time beginning when the preflush weights were taken and continuing until the animals were bred was used as an indication of their nutritive state at the time of breeding. The standard partial regression of ovulation rate on preflush weight was highly significant (0.26, $P<0.01$) whereas the standard partial regression of ovulation rate on average daily gain from the time the preflush weight was taken until breeding was negligible (—0.03). This indicates that the way in which the animal grows out has a greater effect on the number of eggs she will produce than her nutritive state at the time of breeding, at least within the limits of the feeding practices under test.

Ovulation rates for the FF and LL groups were adjusted for the regression of ovulation rate on preflush weight within breed, feed and year. When this was done significant differences between these two feeding groups no longer existed. Thus the significant difference in ovulation rate between these feed groups seems to be fully explained by differences in body weight at the time of breeding. There is, therefore, no evidence that other factors such as changes in nutritive state of the animal, were of real importance in the comparison of the FF with the LL groups in determining ovulation rate. That this may not always be the case is indicated by the fact that ovulation rate was increased by flushing in 1957 without body weight being changed appreciably.

From this study it appears that for yearling ewes the way they grow out is of more importance in determining their ovulation rate than their nutrient supply at the time of breeding, at least for animals continued on the same level of feed as that on which they were grown out. This does not seem to be true of animals which have been flushed and may not be true if the feed level is lowered just prior to breeding. This latter point has not been tested but information concerning it may help in determining further the effect of the nutritive state on ovulation rate in those animals grown out on grain. The relationship might also be different in the case of mature ewes where changes in body weight due to feed would result primarily from differences in degree of fatness and thus the nutrient supply or some factor associated with fatness might be implicated.

Embryo Survival. Data on embryo survival rate for each ewe determined at either 25 or 140 days were pooled in both 1956 and 1957. Inspec-

TABLE 5. PERCENT EMBRYO SURVIVAL[a] CLASSIFIED BY LEVEL OF FEEDING (40 DAYS, 1955; 25 AND 140 DAYS POOLED FOR 1956 AND 1957)

Year	Level of feeding				Total
	LLL	LFL	FFL	FFF	
1955	50.0	40.4	60.7	45.1	49.0
1956	71.4	64.6	....	70.1	68.7
1957	84.0	76.9	....	61.7	74.7

[a] Percent survival for individual ewes were transformed to angles for analysis. Means were then re-transformed to percentages.
Test of significance: LLL *vs.* FFF, P≅.07 (1957).

tion of means indicated that very little embryonic death occurred after 25 days. The overall means for the two stages of gestation were almost identical.

The embryo survival at 40 days over all feed groups studied in 1955 was 49.0% (table 5). The differences among the different feed groups were not significant. The greatest observed difference occurred between the group which was changed from long-time full feeding to a limited level at the time of breeding and the flushed group (60.7 *vs.* 40.4). The continuously limited and continuously full-fed ewes were similar (50.0 *vs.* 45.1%).

The overall mean embryo survival rate was 68.7% for 1956 and 74.7% for 1957 (table 5). The average difference between breed of sire groups for both years is insignificant (72.0 *vs.* 71.3%, respectively, for Columbia and Hampshire, table 6). This indicates little, if any, additively genetic varia-

TABLE 6. EMBRYO SURVIVAL CLASSIFIED BY BREED (POOLED FOR 25 AND 140 DAYS, 1956 AND 1957)

Classification[a]	Embryo survival
Straight-bred Hampshire	55.8
Straight-bred Columbia	77.6
Both straight-breds	67.4
Both cross-breds	75.8
Hampshire sire	71.3
Columbia sire	72.0
Hampshire dam	60.8
Columbia dam	81.4
Hampshire maternal environment	65.7
Columbia maternal environment	84.6

[a] The classification, both straight-breds, is the average of the two classifications straight-bred Hampshire and straight-bred Columbia; the classifications Hampshire maternal environment and Columbia maternal environment (each breed of dam carrying cross-bred fetuses) are components of the respective breed of dam classifications (each breed of dam carrying both cross-bred and straight-bred fetsuses. The above classifications are thus not independent.
Test of significance: Breed of dam, P<0.01. Breed of maternal environment, P≅0.13.

tion between embryos in survival rate. The significant difference found in embryo survival between the Columbia and Hampshire ewes over both years implies, in the light of no breed of sire effect, that there is a marked difference in maternal influence on survival rate between the breeds (81.4 *vs.* 60.8% for the Columbia and Hampshire dams respectively). The difference between Columbia and Hampshire dams in the reciprocal crosses is 18.9% (84.6 *vs.* 65.7%, $P \cong 0.13$).

No significant effect of feed was shown in 1956 (71.4, 64.6 and 70.1%, respectively, for LL, LF and FF, table 5). In 1957, however, the full-fed group was lower as compared to those limited fed (61.7 *vs.* 84.0%) with the flushed group being intermediate (76.9%). These differences were not significant but when the two straight-fed groups were compared on a single degree of freedom the difference approached significance ($P \cong 0.06$). This tends to differ from the results of the two previous years but agrees with earlier work done over a two-year period by El-Shiekh *et al.* (1955), where those ewes receiving grain suffered greater embryo loss than those on a hay ration. The majority of the difference was found in one year, however. In view of the results from these five years the effect of feed on embryo survival is not clear. Year-to-year variation indicates that some environmental factor associated with year may be affecting embryo survival differentially on the two feed levels. These "year" effects might act in such a way either to increase or decrease embryo survival resulting in the masking of a detrimental effect of full feeding or making full feeding appear detrimental.

Summary

Ovulation rate and embryo survival were studied at slaughter in 128 yearling ewes of mixed breeding in 1955 and in 330 grade Hampshire and grade Columbia ewes in 1956 and 1957 combined. Straight-bred and reciprocal cross-bred fetuses were produced by mating purebred Hampshire and Columbia rams to the grade Hampshire and Columbia ewes. The ewes in each year were subjected to various sequences of alfalfa-grass hay only or the same hay plus grain (full and limited levels of feeding, respectively) from the beginning of the experiment in mid-winter until they were slaughtered in the next fall or winter. Breeding was begun in October.

Those animals that were better grown out as indicated by greater body weight shed the greater number of ova in each of the three years. This was true whether variation in body weight was that which existed between ewes within the breed-feed sub-groups or whether it was produced by long time differences in feeding levels. Flushing (full feeding for approximately 3 weeks prior to breeding) seemed to increase ovulation rate in 1957 only. No appreciable increase in weight was shown, hence temporary improvement in the nutritive state of the animal appears to be responsible.

Virtually all of the prenatal deaths seemed to occur by 25 days after breeding. Full feeding was apparently detrimental to embryo survival in one out of the three years.

A greater number of embryos from Columbia dams survived than from Hampshire dams. This was true whether the fetuses were crossbred or straight-bred and was accounted for almost entirely by a better maternal "environment" provided by the Columbia dams. There was little additively genetic variation between embryos in survival rate.

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REPRODUCTION IN THE YEARLING EWE AS AFFECTED BY BREED AND SEQUENCE OF FEEDING LEVELS. II. EFFECTS ON FETAL DEVELOPMENT [1]

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FETAL development can be affected by the plane of nutrition on which the ewe is kept during gestation (Thomson and Frazer, 1939; Underwood and Shier, 1942; Underwood *et al.*, 1943; Wallace, 1948; Palsson and Verges, 1952; Alexander *et al.*, 1956). This appears to be true, however, only during the later stages of gestation (Wallace, 1948; El-Shiekh *et al.*, 1955) and under conditions of severe restriction of nutrient intake (Wallace, 1948; Wallace, 1951; Papadopalous and Robinson, 1957). There is also evidence that birth weight can be affected by such genetic factors as inbreeding (Hazel and Terrill, 1945; Glembocki and Nahimison, 1945; Terrill *et al.*, 1948), a maternal environment (Hunter, 1956; Asker *et al.*, 1954) and non-additive gene action (Asker *et al.*, 1954).

The purpose of this work was to study fetal development as it might be affected by different sequences of a full and limited level of feeding and two breeds of sire and dam.

Materials and Methods

The design of the experiment, the levels of feeding and their sequences, the breeding groups and the number of animals involved and their management have been outlined previously (Foote *et al.*, 1959).

Data collected on all ewes (at 40 days in 1955 and at 25 and 140 days in 1956 and 1957) included carcass weight and finish grade. In 1956, at 140 days, and in 1957, at 25 and 140 days, carcass length, from aitch bone to anterior edge of first rib, and length and circumference of shin bone (soft tissue removed) were also taken. The reproductive tract was removed from each ewe and examined for anatomical defects and the number of corpora lutea. The weights of the maternal cotyledons were taken in 1955.

The following information was gathered on the fetuses and their membranes and fluids: At 40 days of gestation (1955) and 25 days (1956 and 1957), weight of fetus, crown-rump length of fetus (measured with amnion intact), weight of total membranes and weight of total fluids. At 140 days of gestation (1956 and 1957), sex of the fetus, body weight, body length, width at hips, length and circumference of shin bones (soft

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tissue removed), length and width of head, eye muscle area at region of the last rib (planimeter measurement of a tracing on paper from a frozen section), kidney weight, liver weight, cotyledon weight (separated from maternal cotyledons and clipped from fetal membranes) and membrane weight (cotyledons removed).

The effects of two feeding periods on the course of fetal development were considered, from the beginning of the experiment to 25 days of gestation (early feeding) and from 25 to 140 days of gestation (late feeding). Some groups received grain for only a short period of time prior to breeding and were pooled with the groups receiving a limited level of feeding up to 25 days of gestation.

TABLE 1. AVERAGE MEASUREMENTS OF FETUSES AND RELATED TISSUES[a] AND OF DAMS TAKEN AT 40 DAYS OF GESTATION (1955)

Measurement	Sequence of feeding level[b]		
	LLL plus LFL	FFL	FFF
Fetuses and related tissues:			
Body weight, gm.	5.38	5.58	4.95
Crown-rump length, mm.	42.7	41.8	41.4
Fetal membrane weight, gm.	44.1	50.3	57.3
Fetal fluid weight, gm.	68.8	76.3	83.5
Maternal cotyledon weight, gm.	11.3	15.0	13.5
Dams:			
Carcass weight, lb.	49.6	71.3	86.2
Finish grade, score	5.6	7.9	8.8

[a] All measurements on fetuses and related tissues adjusted to the basis of a single lamb.
[b] L=hay only; F=hay plus a full feed of grain. First letter denotes level of feeding from beginning of experiment to October 1, approximately 7 months. Second letter denotes level of feeding from October 1 to breeding, beginning October 15, and continuing until the ewes were bred; approximately 3 weeks. Third letter denotes level of feeding from breeding to 40 days of gestation.

All fetus records were adjusted to the equivalent of a single lamb (at 25 and 40 days) or to a single female (at 140 days). These adjustments were derived for each sub-group by weighting the deviation from the mean of single lambs or single females by the amount of information available and pooling over all sub-groups. In the case of twins, both members were used to derive the adjustment factor, but only one member, selected at random, was adjusted and used in the final analysis. One degree of freedom was subtracted from the error degrees of freedom for the adjustment. Unless nonsignificance is specified only statistically significant differences will be stated as differences.

Results and Discussion

Fetal Development to 40 Days of Gestation. The sequences of levels of feeding studied in 1955 appeared to have no effect, generally, on the various aspects of fetal development (table 1). The weight of the maternal

cotyledons was heavier in the group of ewes that was switched from full to limited feed at the time of breeding (15.0 gm.) as compared to the continuously full (13.5 gm.) and limited (11.3 gm.) fed groups. A difference which approached significance occurred between the membrane weights of ewes on continuously limited feed and those on full feed, the latter being the heavier (57.3 *vs.* 44.1 gm., $P \cong 0.08$). A similar trend was noted by El-Shiekh *et al.* (1955); they found that full feeding increased chorion weight at 40 days of gestation.

The relationship of membrane weight, fetal fluid weight, maternal cotyledon weight and fetus weight, where all tissues had been adjusted to that of a single fetus, was studied within feed groups by standard partial regression analysis, assuming no heterogeneity of variances or covariances. This was done to determine the relative part these supportive tissues have in determining fetus weight. The assumption made was that variations in each are either direct or indirect causes of variation in fetus weight. The standard partial regression of fetus weight on maternal cotyledon weight was 0.32. Maternal cotyledon weight presumably gives some indication of their capacity to act as a bridge in the transfer of nutrients from dam to fetus. It may give also an indication of the length of time the cotyledons have been functioning, the heavier cotyledons being those which became functional first. The standard partial regressions of fetus weight on membrane weight and on fetal fluid weight were negligible (0.13 and 0.04, respectively).

The standard partial regressions of fetus weight were also calculated within feed groups on the finish grades of the dam (an indication of fatness) and carcass weights of the dams (an indication of general growth, including fatness). Both standard partial regressions were insignificant, being 0.18 for finish grade and —0.06 for carcass weight.

Full feeding increased the carcass weight and finish grade of the dams (table 1).

Fetal Development to 25 Days of Gestation. At 25 days of gestation neither level of feeding nor the breeding affected fetal development consistently when both years were considered together. When considered separately, however, fetal development was indicated to be affected by feed in 1956 and by breed of sire and breed of maternal environment in 1957. There was some indication of a reversal of the direction of the effects of breed of sire, feed level and also breed of maternal environment for the two years, the direction of feed level and maternal environment being opposite to breed of sire in each year. This suggests a cancelling of effects between these two breeds of dams (the difference in contribution to the genetics of the fetus of these two breeds being in the opposite direction to the difference in the maternal environment afforded the fetuses, table 2).

The relationships of membrane weight, fetal fluid weight and fetus weight within breed, feed, and year groups for both singles and twins were analyzed by the method of standard partial regression, assuming fetus

weight to be the dependent variate. The weights of these tissues for singles and twins are 221 and 217 mg. for the fetus, 21 and 21 gm. for the fetal fluid, and 5.4 and 5.4 gm. for the membranes, respectively. The 25-day stage is just prior to the establishment of cotyledonary attachment as a permanent link for transfer of nutrients from mother to offspring. For single fetuses, both membrane weight and fetal fluid weight were shown to be associated with fetus weight (0.48 and 0.27, respectively, when fetus

TABLE 2. AVERAGES AND STANDARD DEVIATIONS OF FETUS MEASUREMENTS TAKEN AT 25 DAYS OF GESTATION CLASSIFIED BY BREED AND LEVEL OF FEEDING (1956 AND 1957 POOLED)

Classification[a]	Body wt., gm.	Crown-rump length, mm.	Fetal membrane wt., gm.	Fetal fluid wt., gm.
Averages:				
Straight-bred Hampshire	219	12.4	5.1	20
Straight-bred Columbia	222	12.2	4.9	20
Both straight-breds	221	12.3	5.0	20
Both cross-breds	228	12.6	5.5	22
Hampshire sire	226	12.6	5.6	21
Columbia sire	222	12.3	4.8	22
Hampshire dam	220	12.4	5.0	22
Columbia dam	228	12.6	5.6	21
Hampshire maternal environment	222	12.4	4.8	24
Columbia maternal environment	234	12.8	6.2	22
Limited feed	213	12.2	5.4	20
Full feed	236	12.6	5.2	22
Standard deviations (within subclasses)	16.16	1.12	1.85	6.95

[a] The classification, both straight-breds, is the average of the two classifications straight-bred Hampshire and straight-bred Columbia; the classifications Hampshire maternal environment and Columbia maternal environment (each carrying crossbred fetuses) are components of the respective breed of dam classifications (each breed of dam carrying both cross-bred and straight-bred fetuses). The above classifications are thus not independent.

weight was taken as the dependent variable). When this relationship was studied between fetuses within twin pairs the standard partial regression of fetus weight on fetal fluid weight was also found to be significant (0.55). The standard partial regression of fetus weight on membrane weight was not significant (0.24). The same relationship of fetus weight to fetal fluid weight seemed to exist between members of twin pairs as between single fetuses (significant standard partial regressions of 0.55 and 0.27, respectively).

Growth of Dam as Affected by Feed and Breed to 25 Days of Gestation. Significant differences in body measurements were shown to be due to breed and level of feeding (table 3). Full feeding produced heavier carcasses than limited feeding when pooled for both years (80 *vs.* 52 lb., respectively) and also higher finish grades (8.8 *vs.* 5.8, respectively).

Hampshire ewes had a higher finish grade than Columbia ewes when considered over both years (7.6 *vs.* 7.0).

Other carcass measurements, studied only in 1957, were also found to be affected by breed and feed (table 3). Carcass length and shin bone circumference were both greater in ewes on full feed (66.8 *vs.* 64.5 cm. for carcass length and 4.7 *vs.* 4.6 cm. for shin bone circumference). Those differences due to feed suggest that the ewes grown out on full feed are larger (at least in terms of skeleton) than those maintained on a limited ration. Columbia ewes had greater length of shin bone than Hampshire ewes (12.9 *vs.* 12.0 cm.; $P<0.01$).

TABLE 3. AVERAGES AND STANDARD DEVIATIONS OF CARCASS MEASUREMENTS OF DAMS TAKEN AT 25 DAYS OF GESTATION CLASSIFIED BY BREED AND LEVEL OF FEEDING (1956 AND 1957)

Classification	Carcass weight, lb. 1956 and 1957	Carcass length cm. 1957	Finish grade, 1956 and 1957	Shin bone circumference, cm. 1957	Shin bone length, cm. 1957
Averages:					
Hampshire dam	69	65.1	7.6	4.7	12.0
Columbia dam	63	66.1	7.0	4.6	12.9
Limited feed	52	64.5	5.8	4.6	12.4
Full feed	80	66.8	8.8	4.7	12.5
Standard deviation (within subclasses)	8.13	2.08	1.14	.235	.633

Standard partial regression analysis was made to examine the effect of finish grade (degree of fatness) and carcass weight on fetus weight as they acted within the breed-feed and year groups. Each of these regressions was small and not significant (0.11 and —0.17, respectively). The simple correlation between these two independent variables was 0.53 ($P<0.01$). In 1957 data were available on carcass length of dam. A similar analysis was therefore made using carcass length and finish grade as independent variables. The standard partial regression of fetus weight on carcass length was —0.11 and on finish grade —0.03 (simple correlation between finish grade and carcass length was 0.36, $P<0.05$). These results appear to agree with those shown for similar relationships at 40 days of gestation in that animal to animal variaton in the carcass development as indicated by finish grade and carcass weight have no demonstrable effect on fetus weight early in gestation.

Fetal Development to 140 Days of Gestation. In both 1956 and 1957 the growth of the fetus appeared to be affected most by the breeding of fetus (breed of sire) and maternal environment (table 4). Straight-bred Hampshire fetuses showed greater body weight (11.6 *vs.* 10.6 lb.), shin bone circumference (3.2 *vs.* 2.9 cm.), and area of eye muscle (0.46 *vs.* 0.39

sq. in.) than the straight-bred Columbia fetuses. The latter fetuses were larger in regard to shin bone length (8.6 *vs.* 7.9 cm.). Fetal cotyledon weights from straight-bred Columbia ewes were greater in both years than from straight-bred Hampshire ewes (345 *vs.* 286, $P<0.05$).

Hampshire sires produced heavier fetuses than the Columbia sires. Their fetuses also had greater head width, shin bone circumference, hip width ($P\cong0.06$), and greater area of eye muscle. The Columbia sired fetuses, however, had longer shin bones. There was no consistent effect of breed of sire on either body length or head length. The visceral organs measured (kidney and liver) and also the fetal membranes and cotyledons were likewise apparently unaffected. The effect of breed of dam was not striking (table 4). Fetuses from Hampshire ewes had a greater area of eye muscle in 1957.

As in the case of breed of sire the Columbia dams produced fetuses with greater shin bone length than the Hampshire dams. The fetal cotyledons produced in the Columbia dams were heavier than those produced in the Hampshire dams.

The maternal environment provided by the Columbia dams appeared to be superior, generally, as compared to that of the Hampshire dams. The body weights were 11.3 *vs.* 10.6 lb., ($P\cong0.15$) with head width of 7.2 *vs.* 7.0 cm. ($P<0.05$), and shin bone circumference was 3.2 *vs.* 3.0 cm. ($P<0.01$). The additive genetic contribution of the breed of dam to its offspring as estimated from the assumed equivalent effect of breed of sire can be compared with the measured effect of breed of maternal environment. Pooled effects over both years (Columbia minus Hampshire) for two factors respectively are as follows: Body weight, —0.08 *vs.* 0.7 lb.; head width, —0.1 *vs.* 0.2 cm.; shin bone circumference, 0 *vs.* 0.2; hip width, —0.3 *vs.* 0.3. From these comparisons and from the tests of significance for the effect of breed of maternal environment and of breed of sire it appeared that both the genetic contribution of the breed of dam to its offspring and the maternal environment it provides are important in fetal development. The additive genetic contribution furnished by the Hampshire dams (as indicated by breed of sire) resulted in larger fetuses than that furnished by the Columbia. The maternal environment afforded by the Columbia ewes, however, produced larger fetuses than that afforded by Hampshire ewes. Thus it appears that these contributions of the breed of dam to their offspring tend to cancel each other. This would explain the lesser net effect for breed of dam on fetal development than the breed of sire.

Such a relationship suggests that a negative genetic correlation exists between these two genetic traits. This is in agreement with the work of Bradford *et al.* (1958) who found a similar negative relationship between maternal effects and additive genetic effects for different lines of swine for individual pig weight at weaning and at 154 days of age. This same phenomenon has also been reported in earlier work by Dickerson (1947) and Hazel (1954) in swine and Koch and Clark (1955) in beef cattle.

TABLE 4. AVERAGES AND STANDARD DEVIATIONS OF FETUS MEASUREMENTS TAKEN AT 140 DAYS OF GESTATION CLASSIFIED BY BREED AND LEVEL OF FEEDING (1956 AND 1957 POOLED)

Classification[a]	Body wt., lb.	Body length, cm.	Head width, cm.	Head length, cm.	Shin bone circum., cm.	Shin bone length, cm.	Hip width, cm.	Eye muscle area, sq. in.	Kidney wt., gm.	Liver wt., gm.	Coty-ledon wt., gm.	Mem-brane wt., gm.
Averages:												
Straight-bred Hampshire	11.6	48.0	7.0	9.8	3.2	7.9	8.3	.46	25	167	286	236
Straight-bred Columbia	10.6	48.8	7.0	9.6	2.9	8.6	8.2	.39	26	154	345	239
Both straight-breds	11.1	48.4	7.0	9.7	3.0	8.2	8.2	.42	26	160	315	248
Both cross breds	11.3	48.4	7.0	9.8	3.0	8.3	8.0	.42	26	160	305	230
Hampshire sire	11.4	48.5	7.1	9.8	3.2	8.0	8.3	.45	25	164	300	235
Columbia sire	10.6	48.4	7.0	9.6	3.0	8.4	8.0	.40	26	158	318	233
Hampshire dam	11.0	48.0	7.0	9.7	3.0	8.1	8.1	.44	26	163	288	232
Columbia dam	10.9	48.9	7.1	9.8	3.0	8.4	8.2	.42	26	158	330	236
Hamp. maternal environment	10.6	47.9	7.0	9.6	3.0	8.3	7.9	.42	26	160	292	228
Col. maternal environment	11.3	48.9	7.2	9.8	3.2	8.2	8.2	.44	25	161	315	234
Early limited feed	11.0	48.4	7.0	9.8	3.1	8.2	8.2	.43	26	158	304	236
Early full feed	10.9	48.4	7.0	9.7	3.0	8.3	8.0	.43	25	163	314	231
Late limited feed	10.9	48.3	7.0	9.7	3.1	8.2	8.0	.43	26	158	327	240
Late full feed	11.1	48.6	7.0	9.8	3.0	8.2	8.2	.43	26	164	292	228
Standard deviations (within subclasses)	1.57	2.38	.359	.612	.239	.469	.194	.0722	4.61	38.6	77.5	47.1

[a] See footnote for table 2.

The level of early feed (from beginning of the experiment to 25 days of gestation) was not shown to have an effect on fetal development. Limited late feeding (from 25 to 140 days of gestation) tended to produce heavier cotyledons than full feeding during the same period (327 *vs.* 292, P≅0.06).

In trying to determine the way in which the amount of available nutrients affects the developing fetus it seems necessary to consider what homeostatic mechanism might be involved. Some of the results of this study suggest that supporting tissues such as the maternal cotyledons and fetal membranes may play an important part. At 40 days of gestation the weight of the maternal cotyledons was heavier in those ewes changed from a full to a limited ration at the time of breeding than for either straight full or straight limited fed ewes. At 140 days of gestation those animals which had received a limited level of feed (either from the beginning of the experiment or from 25 days of gestation) had heavier fetal cotyledons than those on full feed for the same periods. It seems logical to consider that these tissues (maternal cotyledons early in gestation and fetal cotyledons at later stages) undergo a compensatory hypertrophy in the case of a shortage of circulating nutrients, resulting in the fetus being better able to compete with the maternal tissue.

Early limited feeding tended to give heavier membrane weights than full for fetuses sired by Hampshire rams (246 *vs.* 222 gm.) and lighter weight membranes than full for fetuses sired by Columbia rams (228 *vs.* 238 gm. P≅0.09).

There was no indication that differences between limited fed and full fed were greater for those tissues or organs considered as late developing, i.e., kidney and liver as compared to those considered as early developing, such as the skeleton (Barcroft, 1946).

Analysis by standard partial regression was again used (as at 25 and 40 days of gestation) to study the relationship of fetus weights and the supporting tissues (fetal cotyledon weight and membrane weight, assuming fetus weight to be the dependent variable). The analysis was made on the basis of fetus to fetus variation in the case of singles and pair to pair variation and also fetus to fetus variation within pairs in the case of twins. Average weights for singles and twins, respectively, were 11.2 and 9.4 lb. for fetus weight, 313 and 240 gm. for cotyledon weight and 244 and 188 gm. for membrane weight.

Fetal cotyledon weight might be expected to play a dominant part in this relationship because of the direct contact of the cotyledons with the dams acting as a link between dam and fetus for transfer of nutrients. Membrane weight may be considered both as a storehouse for nutrients received from the dam as well as an organ of synthesis in converting some of these nutrients to forms better utilized. Its weight also is affected by the vascular supply it carries. Fetal cotyledonary development may affect the degree of development of the membranes which in turn may affect

fetal development; this constitutes an indirect effect of fetal cotyledon weight.

For single fetuses the standard partial regression of fetus weight on fetal cotyledon weight was 0.52 ($P<0.05$) and the standard partial regression of membrane weight on fetal cotyledon weight was 0.67 ($P<0.01$). The standard partial regression of fetus weight on membrane weight was 0.34 ($P<0.05$). From this point of view, fetal cotyledon weight has an indirect effect on fetus weight by way of membrane weight (0.23, the product of the standard partial regression of fetus weight on membrane weight and of membrane weight on cotyledon weight). This indirect effect on cotyledon weight on fetus weight could account for most of the direct effect shown for membrane weight.

Between twin pairs the standard partial regression of fetus weight on cotyledon weight was 0.56 ($P\cong.07$) and of membrane weight on cotyledon weight of 0.69 ($P<.05$). The standard partial regression of fetus weight on membrane weight was 0.29 (not significant). The indirect effect of cotyledon weight on fetus weight by way of the membranes was 0.20 (0.69 x 0.29); again, accounting for most of the direct effect shown for membrane weight.

When calculated between fetuses of twin pairs the standard partial regression of fetus weight on fetal cotyledon weight was −0.10 (non-significant) and of membrane weight on cotyledon weight of 0.75 ($P<0.01$). The standard partial regression of fetus weight on membrane weight was 0.67 ($P<0.05$). The indirect effect of cotyledon weight on fetus weight as it acts through membrane weight is 0.50 (0.67 x 0.75). The assumption was made in all of the analyses that fetal cotyledon weight works indirectly through membrane weight on fetus weight to the full extent of its association with membrane weight.

Fetus weight was significantly associated with both fetal membrane weight and fetal fluid weight, each independent of the other, at 25 days of gestation. The maternal cotyledons are developing by 40 days; at that time their weight showed a significant association with fetus weight and fetal membrane weight and fetal fluid weight did not. At 140 days those factors associated with the weight of the fetal cotyledons had become important and they exerted a greater effect on fetal development directly than indirectly by way of membrane weight. This was true although the indirect effect was assumed to be to the full extent of the association of cotyledon weight with membrane weight. This relationship was true both for variation between single fetuses and between pairs of fetuses.

Growth of Dam—Effect of Breed and Level of Feed to 140 days of Gestation. Growth of the ewe as indicated by the carcass measurements was affected by both breed and feed (table 5). Differences due to breed were shown consistently over both years in favor of the Hampshire for carcass weight, finish grade, and shin bone circumference. The Columbia ewes had greater shin bone length. Early full feeding increased all of these

traits except shin bone length over early limited feeding. Late full feeding also increased carcass weight, carcass length and finish grade as compared to limited feeding during the same period. These results indicate that full feeding yearling ewes accelerates their development in terms of skeleton and presumably muscle as well as fat.

Various workers have reported that the feed level of the ewe late in gestation can affect fetal development appreciably. Analyses were made to test whether evidences of variation in individual nutritional status of the different ewes within breed-feed-year groups as indicated by carcass characteristics are associated with fetus weight. The estimated carcass

TABLE 5. AVERAGES AND STANDARD DEVIATIONS OF CARCASS MEASUREMENTS OF DAMS TAKEN AT 140 DAYS OF GESTATION CLASSIFIED BY BREED AND LEVEL OF FEEDING (1956 AND 1957 POOLED)

Classification	Carcass wt., lb.	Carcass length, cm.	Finish grade	Shin bone circum., cm.	Shin bone length, cm.
Averages:					
Hampshire dam	78	70.4	8.4	4.92	12.22
Columbia dam	70	71.4	7.0	4.76	13.08
Early limited feed	64	70.0	6.9	4.78	12.60
Early full feed	84	71.8	8.6	4.90	12.70
Late limited feed	65	70.2	7.1	4.82	12.68
Late full feed	84	71.6	8.4	4.86	12.62
Standard deviations (within subclasses)	9.7	3.0	1.14	.225	.56

weight gain from 25 to 140 days of gestation [3] was used as an arbitrary measure of the nutritive state of the individual animal, finish grade at slaughter as an indication of degree of fatness and carcass length at slaughter as a measure of growth. The standard partial regression of fetus weight on carcass weight gain was negligible (—0.03). The standard partial regression of fetus weight on finish grade was —0.15 ($P<0.01$) and on carcass length, 0.36 ($P<0.05$).

From these results it appears that the carcass length of the dam, as indicating the bodily development or state of somatic maturity, is an important factor acting on fetus weight. The degree of fatness as indicated by finish grade serves in some way to retard or suppress fetal growth. The individual nutritive state of the animal during the major part of pregnancy, as measured by carcass weight gain, had no demonstrable effect on fetus weight.

[3] The 25-day carcass weights were estimated from the animals live weights using average dressing percentage obtained from a comparable group of ewes killed at 25 days of gestation. Finish grade and carcass lengths were the actual measurements taken when the animals were slaughtered at 140 days of gestation.

Summary

Fetal development was studied at 40 days of gestation in 128 yearling ewes of mixed breeding in 1955, and at 25 and 140 days of gestation in 330 grade Hampshire and Columbia ewes in 1956 and 1957 combined. Straight-bred and reciprocal cross-bred fetuses were produced by mating purebred Hampshire and Columbia rams to the grade Hampshire and Columbia ewes. The ewes in each year were subjected to various sequences of grass-alfalfa hay only or the same hay plus grain (full and limited levels of feeding, respectively) from the beginning of the experiment in mid-winter until they were slaughtered the next fall or winter. Breeding was begun in October.

Full fed ewes showed greater somatic maturity as indicated by carcass weight, finish grade, carcass length and shin bone circumference than limited fed ewes. These levels of feed had no consistent significant effect on fetal development at any of the stages studied.

Fetal development at 140 days was measured in terms of fetus weight, fetus length, head length and width, shin bone length and circumference, hip width, area of eye muscle, kidney weight and liver weight. There was no evidence of a differential effect due to feed on these different tissues or organs.

Variations in the individual carcass measurements of the ewes were associated with variations in fetal development at 140 days. The ewes with longer carcasses (indicating greater development) produced heavier fetuses while those with higher finish grades (indicating greater fatness) produced smaller fetuses. This relationship did not exist at either 25 or 40 days of gestation.

Significant increases in maternal cotyledon weight due to changes from full to limited feeding were found at 40 days. Similar changes in fetal cotyledon weight at 140 days were noted due to limited feeding in the late part of gestation. The increased amounts of these tissues may have acted to obtain a greater nutrient supply from the mother, thus protecting the fetus against a nutrient shortage imposed by limited feeding of the mother. The Columbia dams provided a better maternal environment than the Hampshire. The additive genetic contribution of the Hampshire parents, however, produced larger fetuses than that of the Columbia. The maternal environment had a cancelling effect on the additive genetic contribution with the net effect for breed of dam for various characteristics tending toward zero. Fetal membrane weight and fetal fluid weight at 25 days of gestation were significantly associated with fetus weight. The weight of the developing maternal cotyledons was significantly associated with fetus weight, independent of fetal membrane and fetal fluid weight at 40 days. At 140 days of gestation, fetal cotyledon weight had a significant direct effect on fetus weight which was greater than its indirect effect through

fetal membrane weight. This was true for variation between single fetuses as well as between twin pairs of fetuses.

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PROCEEDINGS OF THE 50th ANNIVERSARY MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

The meeting was held in the Hotel Sherman, Chicago, on November 28–29, 1958, the general session convening at 11 a.m. the first day and divided sessions for discussion and presentation of research papers in Breeding and Genetics, Extension, Meats, Nutrition, Pasture and Forage, Physiology and Teaching being held throughout the day and evening of both days.

"Fifty Years of Progress in Animal Science" was the theme of the meeting. Programs were provided with a gold colored cover as was the November issue of the Journal of Animal Science. Persons registering were given a gold colored metal paper weight and a set of abstracts of the research papers being presented; the latter with indices were bound in the November issue, Volume 17: 1136–1251.

The Golden Anniversary Dinner was held at 6:00 p.m., November 28, with W. P. Garrigus presiding. Oliver S. Willham, President, Oklahoma State University, was guest speaker. The annual business meeting with W. M. Beeson presiding was held after the dinner.

The significance of the 50th Anniversary Meeting and special events in connection with it were described at the general session by the president of the Society, W. M. Beeson.

GENERAL SESSION

Introductory Remarks

W. M. Beeson, *President*

Members of the Society and Guests:

It is a real honor and privilege to welcome you to the 50th Annual Meeting of the American Society of Animal Production. This year we are celebrating our "Golden Anniversary" to commemorate "50 Years of Progress in Animal Science". The entire membership of the Society can be proud of the steady growth and achievements in the fields of extension, research and teaching. Working together from a sound beginning and excellent guidance from the officers and members, the American Society of Animal Production has become a powerful influence in shaping the pattern of the livestock industry. No group has contributed more to the efficient production of meat animals than this Society. Teachers and scientists have worked together for a common goal of improving the gain, feed efficiency, reproductive capacity and carcass quality of livestock.

This Society has grown in 50 years from a beginning of 33 charter members on November 28, 1908, to a membership of 1800 on November 28, 1958. Attendance at our annual meeting has increased to about 1200 registered participants.

In a brief span of 17 years the Journal of Animal Science has increased in size from 368 pages in volume 1 published in 1942 to 1265 pages in volume 17 published in 1958. Approximately 3300 copies of each number of volume 17 were sent out to members and subscribers throughout the world representing 52 foreign countries. The Journal has grown with scientific distinction under the leadership of its editors: R. W. Phillips, 1942–49; L. E. Casida, 1950–54; J. K. Loosli, 1955–57; and W. D. Gallup, 1958.

Many special items have been planned for our "50th Anniversary Year". I would like to discuss these briefly.

No doubt you have seen the November 1958 issue of the Journal of Animal Science. In this 50th Anniversary issue we have attempted to review the progress that has been made in the past 50 years in areas under the following headings: (1) Growth of the Society, (2) Animal Breeding, (3) Nutrition, (4) Physiology, (5) Meats, (6) Livestock Judging, (7) Extension, (8) Teaching, and (9) the Future of Animal Science. It is our hope that these articles will act as a stimulus and guide to those engaged in extension, teaching and research. In my estimation this is the best collection of review articles that has ever been published in a single journal covering the various phases of Animal Science. Dr. J. K. Loosli, as Editor of the review papers, and all the authors are to be highly commended for a job well done.

On Friday evening, we are holding a "Golden Anniversary" banquet in the George Bernard Shaw Room. At this banquet we are honoring the two living charter members of the Society—Dr. E. B. Forbes of Pennsylvania State University and Professor H. R. Smith, formerly with the University of Nebraska. Nineteen Past Presidents of the Society are to be seated as guests at a special table.

This year the Society has established the title of Honorary Fellow for members who have rendered distinguished service in the field of Animal Industry over a period of at least 25 years. One person per year may be selected from each of the five following categories: teaching, research, extension, administration and industry. In addition, not to exceed three Honorary Fellows may be selected irrespective of category. Fellows will receive an enscribed scroll and a life membership in the Society. This gives the Society an opportunity to honor individuals who have made a distinguished and lasting contribution to the Society and to the Livestock Industry.

Action is being taken by the Executive Committee to establish an Extension Award and a Teaching Award of $1000 each to honor those individuals who have made outstanding contributions in these fields.

Living exhibits are set up at the International Amphitheatre to show "50 Years of Progress in Animal Science". The name and sponsor of each exhibit are as follows:

50 Years of Progress in Animal Nutrition

1908 vs. 1958 Ration

1. Identical Twin Beef Steers—W. M. Beeson and T. W. Perry, Purdue University.
2. Fattening Lambs—U. S. Garrigus, University of Illinois.
3. Finishing Hogs—R. J. Meade, University of Minnesota.

50 Years of Progress in Animal Breeding

1908 vs. 1958 Type

4. Beef Cattle—Doyle Chambers, Oklahoma State University.
5. Sheep—O. D. Butler, Texas A & M College.
6. Swine—L. N. Hazel, Iowa State College.

50 Years of Progress in Meats

7. 1908 Meat Counter vs. 1958 Meat Counter—Carl Neumann, National Live Stock and Meat Board, and L. E. Kunkle, Ohio State University.

The International Live Stock Exposition has given the Society a prominent place to display this exhibit, and there is a good possibility that this exhibit will be televised on the Arthur Godfrey Show. The Exhibit Committee was composed of T. W. Perry, Purdue, Chairman, W. W. Green, University of Maryland, and O. D. Butler, Texas A & M College. The beef nutrition part of this exhibit is now on display in the lobby of the Sherman Hotel.

National Animal Science week was declared by the governors of Colorado, Texas and Washington to commemorate "50 Years of Progress in Animal Science".

A. E. Darlow, Dean of the College of Agriculture and Vice-President of Oklahoma State University, has been chosen as the 35th honor guest of the American Society of Animal Production. Dr. Darlow's portrait will be presented to the Saddle and Sirloin Club at a banquet in his honor at 6:00 p.m. on Sunday evening, November 30, 1958. Professor Glen Bratcher, Head of the Department of Animal Husbandry, Oklahoma State University, has served as chairman of the Darlow Portrait Fund and arranged the program for the honor dinner.

It has been a real pleasure and inspiration to me to have served the Society as its Secretary-Treasurer and Business Manager for three years, as its Vice-President for one year, and during the past year as its President. Personally, I want to thank all the officers, directors, committee members and others for their fine support and cooperation during my tenure of offices. It has been a privilege to work with you as an officer in my various capacities over the past five years. I trust that the next 50 years of this Society will be as fruitful as the past 50 years.

LOOKING INTO THE FUTURE

R. M. BETHKE

Ralston Purina Company

No one knows better than the individuals in this room that agriculture—whether animal or otherwise—is in a state of dramatic change or progress. Specialization, larger units and greater volume are the order of things.

In the 50 years this Society has been in existence, animal agriculture has transcended from the non-scientific—or more or less art stage—into a scientific profession or business; from the general farm to the specialized farm—from horsepower to mechanical power—from the time one farm worker supported about seven other people to the present when one farm worker supports better than twenty-three other persons; when the farm population made up at least one-third of the total population to where it is somewhat less than 12 percent of the total today—and tomorrow it will be still less.

The past and present members of this Society had an important part in these changes. They and you—directly and indirectly—accounted for the great advances in breeding, feeding and management practices. They and you have played a major role in training and directing the extension specialists who have played such a vital part in the progress of animal agriculture. Yes—you have a big stake in providing the average American and Canadian family with the best protective foods— quality-wise and quantity-wise. Truly you can be proud of your accomplishments of the past half-century.

Today we are at a crossroad as we take inventory of our teaching, research and extension programs. Today you are training youth for jobs which in many respects have different requirements than they had 25 or even 10 years ago. In fact the number and kinds of jobs for your graduates have changed greatly. Actually "your boss", the animal production man of today or the future, is a different type of an individual in that his thinking, his job requirement, and his investment is more exacting and greater than a decade or more ago.

For many years the employees of my own company have been continually reminded that their "Boss" is the farmer or feeder whom they serve. To visualize this, a photograph of a typical farmer was chosen to typify the "Boss". The photograph is set before employees in meetings and around the offices. Here's the picture that was used for many years, starting in the early 1920's. Something about this man seemed to symbolize the farmer of his era. He was a man who did most of his own labor. He usually did it the hard way, without benefit of today's modern labor-saving machinery. He

was largely a general farmer. He had a few hogs, a few cattle, a few layers, milked a few cows and raised much of his own grain.

As times changed on the farm, our image of the "Boss" also changed. We had to throw away this picture of a farmer of another era and symbolize the farm operator of the 1950's. Here is the picture that we use today. The "Boss" is a specialist, a mass producer, a businessman, a shrewd operator who is eminently well informed in his field.

The question of interest here is; To whom will this specialist go for counsel in the future? What kind of research will be of most value to him? What manner of college curricula will best train young men to become the "Boss" and the service men to the "Boss".

In the field of teaching, several agricultural colleges are keeping abreast of these changing times. They are modernizing their curricula to embrace the wide field of agriculture in two broad classifications, "Agribusiness" and "Agriscience."

This is a necessary step in the field of teaching if colleges are to perform their greatest service in the future. It is imperative that the future farm operator and individuals calling on him receive training in the business aspects of farming or livestock production as well as in the science of production. Huge capital investments are involved in individual farms today. Good farm operators measure their income in terms of return on investment, just as industry and business have done for a long time. Competent money management has become as important to good farming and livestock production today as are modern production techniques.

In my judgment, all agricultural colleges will have to modernize their curricula to embrace the changing trends to justify their existence. It is difficult to see how agriculture can be taught to prospective farm operators in this modern era in the absence of an all-inclusive concept of the subject matter.

I was on the academic scene long enough to have learned that curricula are not overhauled easily and quickly. It is a major undertaking to revise the concept of broad subject matter. It involves adjustments in personnel as well as adjustments in subjects and subject matter. It won't happen overnight; but if colleges are to perform maximum services in the future, and I am sure they will, they will have to keep their teaching abreast of the changing times.

The same general philosophy reaches into research. There is no doubt that research in our agricultural colleges, experiment stations and Government agencies has performed a remarkable service for agriculture. Most of the modernization of our breeding, feeding and production methods is the direct result of their research.

The all important question facing researchers and research administrators of today and the future is; Where should the emphasis on research be placed? By this question, I do not mean to infer that it is a question of

basic versus applied or fundamental versus practical—but rather how much basic and how much "products testing" type work.

Basic or fundamental research is the foundation of progress. It must never be neglected.

In the past, in my judgment, research of the "products testing" type frequently had greater emphasis than basic or fundamental research. There were good reasons for this—including financial support. However, I wonder whether we have not reached the point where greater emphasis needs to be put on basic or fundamental research and less on straight products testing. Most industries serving agriculture have over the past one or two decades, greatly expanded their research and testing facilities as well as their technical staff and are well equipped to do "products testing work". In view of these considerations, is it necessary and are you justified in spending your time and using your facilities for this type of work? In raising this issue, I do not mean to infer or imply that I question the propriety of industry supporting research at colleges or experiment stations. Actually, I am in favor of industry supporting college or experiment station supervised research. The issue to my way of thinking is: What is the purpose of the grant or support—realizing that results or data can be used in various ways.

This leads me to the next question; Where will the Extension man find his greatest usefulness in the future? This question is worthy of serious thought. Today there are some 4 million 800 thousand farmers in the United States. However, about 85 percent of the gross income from all farm production is produced by only 15 percent of the total number of farms. This shows in a realistic way what we mean when we say that the modern farmer is a big business man.

To whom will this big operator look for counsel and information? Who can command his complete confidence? Is this well informed production specialist becoming self-sufficient, or does he still need the kind of services that extension departments offer?

Possibly the services that the extension man *can* perform best in the future might be brought into focus by first pointing out the services that he ordinarily will *not* be able to perform best. As an example, he won't be able to offer basic services in the field of breeding and genetics. The big commercial breeders have developed this field to the point where large operators do not depend on them for basic information. Similarly, the extension man generally is not a nutrition specialist. The big feeder has made himself virtually a specialist in feeds. He frequently depends on a commercial feed company representative in whom he has confidence. There are other similar specialized fields where industry has performed a service to agriculture in such a magnitude that it has rightfully won the feeders' confidence.

This does not mean to imply that the extension man should exclude himself from service in these fields. However, he will have to adjust his

concept of his job on a realistic basis and operate accordingly. He will have to be thoroughly informed on the commercial as well as the college developments so that he will be a valuable advisor and source of information for the professional farm operator and to industry serving agriculture. The best librarian is not the one who attempts to know the detailed contents of every book on the shelves. He is the one who knows which books contain accurate and dependable information on the subject at hand, and he knows where to find it.

The extension service can continue to be of genuine usefulness to the new "Boss". Extension men and leaders will need to recognize changing trends and adjust to them. They can increase their effectiveness by accepting and understanding the role of industry as a partner in serving agriculture. They will, in my judgment, have to recognize that the primary function of extension service is one of learning basic problems of individual operators and serving these operators by helping to guide them to practical solutions, wherever the solution to the problems may lie.

College teaching, research, extension and industry, all serve the same master or boss. This master or boss is the American farmer and his business associates. Each one, whether it is teaching, research, extension or industry, serving the master or boss has a function to perform and each one can perform it best by cooperating with the others.

No field of American enterprise has progressed more rapidly in the past decade or so than has agriculture. This progress has been due largely to ever-improving team work between all those serving agriculture. I am confident that this team work will become strengthened and will manifest itself even more in the years ahead, leading to greater progress and usefulness for all concerned.

A SUMMARY OF THE MINUTES OF THE FIFTIETH BUSINESS MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

H. H. STONAKER, *Secretary*

The meeting was called to order in the George Bernard Shaw Room of the Hotel Sherman, Chicago, Illinois, on November 28, 1958, at 8:35 p.m. by President W. M. Beeson. The minutes of the 1957 Business Meeting were not read but were approved as published in the Journal of Animal Science, Vol. 17, No. 1, pp. 254–265.

The report of the Secretary was as follows:

"Membership in the American Society of Animal Production increased to 1829 in 1958. There were 264 new members with a net increase of 127 in total membership. The membership is international in nature with the following distributions: The U.S.A. and Canada, 1751; other countries, 78. Of the foreign memberships, 22 were assisted by the International Cooperation Administration.

"Subscriptions to the Journal of Animal Science increased by 52 to reach a total of 1434. Five hundred and eighty-two Journals go to the U.S.A. and Canada, 852 go to foreign countries.

"There were 230 papers accepted for presentation and 28 papers to be 'read by title' for the 1958 program. In addition there were three invitational papers in Teaching and four in Extension Sections. An additional section has been incorporated into the 1958 meetings which has made it possible to include 65 more papers than were presented in 1957 and a decrease of 34 in the number 'read by title'."

The Secretary's Report was approved as read.

The Treasurer of the Society, H. H. Stonaker, presented the following report:

"As of October 31, 1958, the American Society of Animal Production had total assets of $46,481.37. Of this total, $6,615.99 were in demand deposit, $21,130.88 were in Savings Deposit and $18,734.50 in U. S. Savings Bonds. The balance in the Morrison Award Fund was $4,468.14, leaving $42,013.23 in the General Society Fund.

"Expenditures in 1958 were $32,348.06. Income in 1958 was $33,124.22 with an excess of income over expenditures of $776.16.

"Publication cost of the Journal was the major source of increased expense in 1958."

The Treasurers report was accepted as read.

President W. M. Beeson read part of the Auditors Report and stated that the detailed records are on file in the Treasurers Office for examination. The audit for the year ended October 31, 1958, was made by the firm of Rogers & McCluskey, certified public accountants, Fort Collins, Colorado. Dr. H. M. Briggs made the motion to accept the auditors report, D. F. Stephens seconded the motion. The motion carried.

The Business Manager's Report was as follows:

"Three thousand five hundred Journals were printed quarterly with an average total cost of $1.71 per copy. Three thousand two hundred sixty-three are being mailed to members and subscribers.

"The records of the Business Manager listed the following back numbers of the Journal and proceedings available for sale:

JOURNAL OF ANIMAL SCIENCE

Vol.	Year	Complete volumes	Number of copies in single issues			
			No. 1	No. 2	No. 3	No. 4
1	1942	0	2	0	0	0
2	1943	0	7	0	0	0
9	1950	0	0	0	34	37
10	1951	0	0	79	0	28
11	1952	0	0	0	57	115
12	1953	7	28	63	48	7
13	1954	241	241	262	279	343
14	1955	17	45	60	69	17
15	1956	20	20	66	90	417
16	1857	163	163	164	179	171
17	1958		335	185	214	

PROCEEDINGS OF THE SOCIETY

Year	No. of copies	Year	No. of copies
1915–16	41	1931	53
1922	55	1932	14
1923	48	1933	33
1925–26	37	1934	20
1927	48	1935	23
1929	24	1940	38
1930	24		

"These back numbers may be purchased from the Business Manager at the following rates: Volumes 10–17—$2.00 per copy; preceding issues—$1.25 per copy; Proceedings—$1.00 each. Please add 25 cents for all foreign deliveries."

The Editor of the Journal of Animal Science, W. D. Gallup, gave the following report:

"The number of manuscripts published in a single volume of the Journal of Animal Science has more than doubled in the past 10 years. The number of pages per volume has increased from 568 in 1948 to 1265 in 1958. A 10-year summary of publication records taken from editor's annual reports shows that 63 manuscripts and 126 abstracts were published in 1949, as compared with 130 manuscripts and 258 abstracts in 1958.

JOURNAL OF ANIMAL SCIENCE PUBLICATION RECORD [a]
1949–1958

Vol.	Year	Mss.	Abstr.	Pages [b]	Vol.	Year	Mss.	Abstr.	Pages
8	1949	63	126	665	13	1954	112	200	1048
9	1950	85	144	694	14	1955	129	211	1274
10	1951	118	149	1098	15	1956	138	241	1318
11	1952	80	164	818	16	1957	126	246	1130
12	1953	104	156	968	17	1958	130	258	1265

[a] See J. Animal Sci. 8:148 (1949) for record from 1942 to 1949.
[b] Pagination includes occasional symposia, professional addresses to the Society, summary of minutes of annual business meeting, biographical sketches and pictures of award recipients and honor guest, memorials, news and notes of interest and annual indexes.

"During the past 12-month period, from November 15, 1957, to November 15, 1958, a total of 227 manuscripts were received for publication. Of these, 63 had been published, 58 are in press for the February 1959 issue, 42 are being revised and edited, 26 are being reviewed by the editorial board, and 38 either have been withdrawn or have been found unacceptable by the review board.

"A similar distribution of manuscripts during the year 1948–49, 10 years ago, shows 72 manuscripts received and 11 rejected: in the year 1953–54, 142 manuscripts received and 13 rejected; and, as stated, in the year 1957–58, 227 received and 38 rejected.

"Credit for successful publication of the November, 1958, issue of the Journal to commemorate the 50th Anniversary of the American Society of Animal Production belongs to J. K. Loosli, former editor, the elected officers of the Society and the Boyd Printing Company. Dr. Loosli has spoken for the entire membership in his expression of appreciation to the authors who prepared the review papers for that issue.

"The sincere thanks of the editor and the associate editor, A. B. Chapman, are extended to C. F. Huffman and S. W. Terrill for the excellence of their work in reviewing manuscripts during the past four years."

DISTRIBUTION OF MANUSCRIPTS RECEIVED FROM NOVEMBER 15 TO NOVEMBER 15 OF THE YEARS 1948 TO 1958

Period	Received	Published	In press	Being revised	Being reviewed	Unacceptable
1948–49	72	27	17	15	2	11
1949–50	129[a]	55	32	25	7	3
1950–51	116	65	21	7	15	8
1951–52	100	45	26	16	4	9
1952–53	127	66	33	10	9	9
1953–54	142	66	33	9	21	13
1954–55	162	95	35	8	12	10
1955–56	153[b]	75	31	10	15	22
1956–57	170	67	33	24	33	13
1957–58	227[c]	63	58	42	26	38

[a] Nine invited papers of two symposia were combined to form two for publicaton, J. Animal Sci. 9:461 (1950) and 10:3 (1951).

[b] Includes four invited papers presented at symposium, J. Animal Sci. 16:224 (1957).

[c] Includes 14 review papers in the 50th Anniversary issue, J. Animal Sci. 17:907 (1958) and three professional addresses, J. Animal Sci. 17:277, 495 and 507 (1958).

The report of the Nominating Committee was given by L. E. Casida, Chairman: W. P. Garrigus was nominated for President; L. E. Harris and J. K. Loosli were nominated for Vice President; H. H. Stonaker was nominated for Secretary-Treasurer. Mr. H. M. Briggs moved that unanimous ballot be cast for W. P. Garrigus as President and H. H. Stonaker as Secretary-Treasurer. M. E. Ensminger seconded the motion. It was carried. W. M. Beeson appointed D. E. Becker, J. A. Hoefer, and W. E. Dinnuson to distribute the ballots and to serve as tellers.

E. J. Warwick gave the following report of the Research Committee:

"Activities of this committee during the past year have been limited to further reviews of monograph material. This work has been nearly completed and we recommend that the Society proceed with publication of the monograph."

The report was accepted.

The Monograph Committee report was given by Clair E. Terrill as follows:

"The Monograph, 'Techniques and Procedures in Animal Production Research', has been completed for some time except for certain revisions. It has been extremely difficult to synchronize the revision of the various sections. It is planned to submit the revised sections for printing soon."

The report was accepted.

The report of the Teaching Committee was given by W. A. Cowan:

"Your teaching committee believes that outstanding teaching deserves recognition by the Society and as a result by all those interested in Animal Agriculture.

"We have drafted a detailed proposed set of rules and regulations that may be used by the Society for such a teaching award. This will be presented to the officers and directors. We believe that the award should carry with it a suitably inscribed plaque and remuneration of at least $1,000 annually. We are proposing that this be called the Distinguished Teacher Award of the American Society of Animal Production. We have listed at least a dozen organizations that might be interested in being the donor of this award. We hope to have an answer in the near future on specifically what this will be.

"We are optimistic enough to believe that this teaching award will be available next year. The program this afternoon in the teaching session concerned itself with (a) the personnel needs and training programs for the feeds industry and (b) the California Polytechnic State College program for motivating students. The problem of improving the quality of instructors and students is constantly before us as is the need for keeping curricula abreast or ahead of the times without having a program that swings from one extreme to another."

The report was accepted as read.

W. P. Garrigus gave the report of the Membership Committee:

"The 1958 Membership Committee consisted of: Prof. George Vander Noot, Rutgers University, Dr. Frank Whiting, Lethbridge, Canada, Dr. A. L. Neumann, University of Illinois, Dr. R. F. Wheeler, Clemson Agr. College, Dr. A. L. Musson, South Dakota State College, Mr. Jerry Sotola, Armour & Company, Mr. Tom Glaze, Swift & Company, and Dr. Wm. H. Burkitt, Montana Flour Mills Co., Great Falls, Montana, and Dr. W. P. Garrigus, University of Kentucky.

"The college personnel were each given the responsibility of contacting department heads in their respective regions as established for the regional research programs. The industrial members were each asked to canvass their representative industries. One of the industry members was successful in inserting notices in Western Feed & Seed, Commercial Review, and Feedstuffs. The above program has resulted in 127 additional memberships for the Society for a total membership as of October 31, 1958, of 1829."

The report was accepted.

The report of the Information Committee was given by Carl F. Neumann:

"It has been a pleasure to again serve in a small way the Society of Animal Production, which has contributed so much to the field of research in animal production and ultimately, of course, to the general welfare of the American consumer—in fact, to consumers of the entire world.

"We are grateful to Dave Thompson of Indiana, who has assisted the committee this year in handling publicity work connected directly with this meeting. Mr. Thompson has manned a press room which served as a clearing house for information to representatives of the press and to radio and television, as well as members of the National Association of Radio and Television Farm Directors, who are currently in session here in Chicago.

"This year reviews were prepared of a number of the special articles in the commemorative issue of the Journal of Animal Science, as well as releases on papers presented at this meeting, and distribution was made to 289 newspapers, livestock journals, industry publications, and 184 radio and television farm directors.

"This material went to such publications as the Corn Belt dailies, St. Joseph Market Journal, American Hereford Journal, American Cattle Producer, Poland China Journal, Montana Wool Grower, National Hog Farmer, Western Livestock Journal, Aberdeen Angus Journal, American Hampshire Association, National Wool Grower, Livestock Weekly Magazine, National Provisioner, Meat Magazine, and the Butchers Advocate.

"The publication list also included such other publications as the Colorado Farmer and Rancher, Nebraska Farmer, Pennsylvania Farmer, Prairie Farmer, and the Farmer Stockman, to name but a few.

"Likewise, information was distributed concerning the '50 Years of Progress in Animal Science' exhibit now in progress at the International. This exhibit, incidentally, will be featured tomorrow on the NBC National Farm & Home Hour by our old friends, Everett Mitchell and Milt Bliss.

"Likewise, publicity releases have been prepared on a number of the papers presented here at this meeting, with distribution going to the Chicago City News Bureau, which services the various press services as well as the City News Bureau here in Chicago and to daily newspapers in Illinois, Kentucky, Texas, Montana, Wyoming, Oklahoma, Missouri, Wisconsin, Arkansas, Indiana, Iowa, Nebraska, California, Tennessee, Colorado, Pennsylvania and Utah, this being a list of papers that have shown a particular interest in publicity concerning your activities in the past.

"Releases concerning the recipients of the American Feed Manufacturer's Association Award, the Morrison Award, and the Society Fellow Awards, were made through the Chicago City News Bureau and its services, who were glad to assist in seeing that this information received local treatment.

"Likewise, information was released on the annual portrait hanging in the same manner.

"Reviews of the above mentioned publications and a look at our various clipping services have shown us that a broad use was made of this material because, No. 1, it concerns information vital to not only the livestock and meat industry and agriculture as a whole, but to the American consumer; and secondly, it is news in the various localities of the country from which this material and information originated.

"The members of your Information Committee are grateful for the opportunity to be of service."

The report was accepted.

H. H. Cole gave the following report for the Committee on Necrology:

"The Necrology Committee recommends that memorials transmitted herewith of the following deceased members be published in the Journal of Animal Science: F. W. Atkeson, Phillip Martin Brandt, Edmund Cheng, Nicholas Ittner, Reuben F. Johnson, Frank B. Morrison, William E. Pugh, William H. Riddell, E. L. Shaw, Charles M. Thompson, Elwin L. Willett and Laurence M. Winter."

A moment of silence was observed in remembrance of these deceased members.

E. W. Crampton submitted the report of the Resolutions Committee instructing the Secretary to write those concerned informing them of the resolutions of the Society.

"The November 1958 number of the Journal is a special issue commemorating the 50th Anniversary of the founding of our Society. While the overall planning of this issue was in the hands of our President and his Executive Committee, the immediate supervision of the assembly of the 14 special articles depicting the growth and development of the animal production industry was in the hands of J. K. Loosli, who acted as 'consulting editor'. In addition, 14 members contributed the papers comprising the story.

"Be it resolved, (1) that our Society greatly appreciates the thought, time and efforts of those who planned and supervised the preparation of this commemorative number of the Journal and that of all who contributed the special papers and be it further resolved that official notes expressing our thanks be sent by the Secretary to those concerned.

"The Committee on Information has been particularly active this past year in publicizing the special articles appearing in the 50th Anniversary issue of our Journal.

"Be it resolved, that a special vote of thanks be tendered to Carl Neumann and his associates, H. H. Bigger and W. D. Gallup of the Information Committee, for their service in making the activities of our Society better known.

"Our thanks are due to Dean H. H. Kildee and the Board of Directors of the International Livestock Association for donating the space at the International Stockyards Amphitheater for the several exhibits illustrating 50 years of progress in Animal Science and to the colleges who supplied the exhibits.

"Be it resolved, that appropriate letters of thanks be sent to the following: H. H. Kildee, International Livestock Association and to the appropriate Departments of: Texas A & M College, Sheep Breeding exhibit; University of Illinois, Sheep feeding; Iowa State College, Swine breeding; University of Minnesota, Swine feeding; University of Oklahoma, Beef breeding; Purdue University, Beef feeding; and to the National Livestock and Meat Board, National Tea Co., and Ohio State University for the meats exhibit.

"Be it resolved, that the American Meat Institute and the Illinois Agricultural Association be commended for their efforts to encourage the use of leather for prize ribbons, awards and souvenirs relating to livestock shows and similar events.

"Our Society greatly appreciates the facilities, courtesies and assistance from the Management of this Hotel in connection with our meetings.

"Be it resolved, that a letter expressing our thanks be sent to Hotel Sherman."

Report of Special Representatives of the Society

T. C. Byerly gave an oral report as the Society's representative with the American Association for the Advancement of Science.

W. M. Beeson in the absence of J. E. Foster, presented the following report on the Scientific Manpower Commission:

"Since the fourth annual report given at the business meeting of the society last year, meetings have been held in Washington, D. C. on February 21, June 24 and November 6. The one in June was a meeting of the Executive Board. At the annual fall meeting on November 6, your representative was renominated for Secretary-Treasurer. The American Society of Animal Production made a contribution of $150.00 to the Scientific Manpower Commission this year.

"At the February meeting the Executive Director reported that the joint EMC-SMC annual letter soliciting contributions from industry had been mailed early in January and that the returns thus far ($10,231.65 for SMC), though not as poor as had been feared, were substantially below those of 1957. In 1957, SMC managed to stay in the black by keeping expenditures well below budgetary limits. At the June 1958 meetings the Executive Director was authorized to draw upon reserves to the extent of but not to exceed $7,500 to defray operating expenses through December 31, 1958.

"A disproportionate amount of time is still being taken up with military problems. Selective Service cases have been increasing, particularly in the graduate student category. Some of these cases have been Animal Husbandry students, from various parts of the country, who contacted your representative. There has also been a larger volume of requests from scientists to assist them in effecting transfers from the ready to the standby reserve. Requests for facts, figures and other information on the scientific and engineering manpower situation have grown in number and have come from government agencies, members of the Congress, Congressional committees, the press, industry and miscellaneous sources. The distribution of career literature also continues brisk, and the SMC brochure has been completed.

"SMC was a co-sponsor with the Office of Education of a conference on February

10 and 11 to consider problems in the teaching of science and mathematics in our public schools. Successive sessions were devoted to teachers and teacher training, teaching methods and teaching equipment. It was also a co-sponsor (with the Society of American Military Engineers) of the Military-Industrial conference, held in Chicago.

"The Commission endorsed in principle the major legislative provisions contained in the Hill-Elliott Congressional Bill dealing with educational matters. Some members of the Commission have been actively engaged in assisting the 'President's Committee on Scientists and Engineers'. This included a series of 'utilization conferences' being held in various parts of the country to consider how scientific and technological personnel can best be utilized by industry. The Commission has been requested to assist with the editing of a proposed volume dealing with Soviet Science, which is under consideration by the Public Affairs Press, of Washington, D. C. The A.A.A.S. volume on this subject is out of print and a new one appears to be desirable.

"The President's Committee on Scientists and Engineers' will terminate its work and its existence on December 13, 1958. Since the Local Action and Utilization Conference projects cannot be terminated by that time the SMC Executive Director was authorized to explore jointly with the Executive Secretary of SMC the feasibility of taking from this Committee the responsibility for continuing these projects and to explore sources of financial support commensurate with the responsibilities assumed.

"There will be a conference on Scientific Manpower held in Washington, D. C. on December 30 in conjunction with the A.A.A.S. convention. The subject for consideration at this conference which will be presided over by the commissions, is 'The Employment Situation for Scientists and Engineers in 1959'."

James O. Grandstaff presented the following report from the Division of Biology and Agriculture, National Research Council and Agricultural Research Institute:

"The 1958 meeting of the Division of Biology and Agriculture was limited to a half-day session on April 1, as part of the first general meeting embracing all eight divisions of the National Research Council. The program of the general meeting dealt primarily with five main topics selected because of their general interest to science and scientists generally and embraced many activities of the Academy Research Council. Topics included the following: (1) Education in Science, (2) International Aspects of Science, (3) Beyond the International Geophysical Year, (4) Information and Documentation in Science, and (5) Problems in the Stimulation of Research. Prominent speakers for each of these topics outlined their broad aspects in terms of current issues, developments and opportunities in this time of rapid scientific advances, which was followed by group discussions.

"The Division meeting devoted considerable time to discussion of its role in biological education. Eleven of the societies represented at the meeting were engaged in some kind of educational program, including accreditation of professional schools. The sentiment of the meeting was against extension of accreditation into biological specialties, but suggested that societies might properly recommend changes in curricula and educational standards.

"The U. S. National Committee of the International Union of Biological Sciences is being reorganized to provide adequate representation of the National Biological societies. Twelve of its 15 members will be selected from nominations made by the various societies represented in the Division of Biology and Agriculture. This committee will be concerned with making nominations of delegates to the General Assembly of IUBS, and recommendations relating to U. S. participation in other international meetings.

"Chairmanship of the Division passed from Dr. L. A. Maynard to Dr. H. Burr Steinback on July 1, 1958. Membership of the Division now consists of 10 members-at-large, 3 members representing certain government agencies, and 34 representing national biological societies. There are also 4 so-called liaison members from other government

agencies. Dr. Grace Goldsmith has been appointed chairman of the Food and Nutrition Board, succeeding Dr. Conrad Elvehjem.

"The following summarizes the current status of reports of committees of the Agricultural Board, NAS-NRC, of interest to society members:

"1. *Committee on Animal Nutrition:* Nutrient Requirements of Beef Cattle, revised 1958, NAS-NRC publication 579. Other reports and revisions now in progress include nutrient requirements of poultry, swine, horses, and laboratory animals; Fluorosis problem in livestock production; public health aspects of feed adjuvants; and a report on joint U. S.-Canadian tables on feed composition.

"2. *Committee on Animal Breeding:* In addition to developing a report on lines of genetic research that appear most promising, consideration is being given to problems relating to irradiation in animals, physiology of farm animals at high altitudes, breeding for efficiency in terms of meat made per unit of feed eaten, selection of breeding stock on the basis of results of performance testing stations, maintenance of a control flock or herd in order to measure genetic progress and how to facilitate importation of valuable breeding stock.

"3. *Committee on Feed Composition:* issued a 661-page volume on 'Composition of Cereal Grain and Forages' as NAS-NRC publication 585. The Committee was released as of July 1, 1958. The Agricultural Board has received numerous requests for continuation of this work but thus far does not have the necessary financial support.

"4. *Committee on Range and Pasture Problems* is nearing completion of the Range Research Methods and Techniques handbook in cooperation with the American Society of Range Management.

"5. *Committee on Animal Health* has been reorganized. The subcommittees on Calf Losses and Losses in Young Pigs expect to consider genetic, nutrition, management, and disease aspects.

"6. *Committee on Agricultural Pests* is working on the development of a coordinated program for evaluation of losses caused by plant and animal pests, and for an analysis of the research required to develop effective and economic controls.

"7. *Committee on Agricultural Engineering* issued a report on 'Research Needs Relating to Agricultural Equipment'. Emphasis was placed on the need for coordination of biological and engineering research. Consideration is being given to other proposals including application of electronics and ultra-sonics to the solution of agricultural problems, and animal environmental control with special reference to animal production in hot climates.

"8. *Committee on Fats and Oils* is developing a report on the interplay of factors which determine the production, marketing, pricing and consumption of fats and oils in relation to the overall agricultural economy.

"In the past year applications for National Science Foundation fellowships in the Life Sciences, screened by committees of the National Research Council, included 882 predoctoral, 205 postdoctoral, and 118 senior postdoctoral. Fellowship awards by National Science Foundation in fiscal year 1958 included 5 predoctoral and one postdoctoral in agriculture. Fellowships awarded in the fields of genetics, biochemistry, general biology and zoology total 100 predoctoral, 12 postdoctoral, and 13 senior postdoctoral. American lecturers and research scholars receiving U. S. Government awards under the Fulbright and Smith Mundt Acts for the 1958–59 academic year included four scientists in animal and dairy husbandry.

"The seventh annual meeting of the Agricultural Research Institute was held October 13–14, 1958, at the National Academy of Sciences. The ARI supports the Agricultural Board by serving as forum for the discussion of problems of broad significance to agriculture, and recommending activities which the Agricultural Board might undertake, as well as providing its principal basic financial support. This year's program included panel discussions on (1) Problems Relating to Agriculture in the Fertilizer Producing Industry, (2) Public Relations in Agriculture and Agriculture Research, (3) Mobilizing Research for Progress in Soil and Water Conservation. Addresses on other topics of broad interest were given by Dr. R. W. Phillips,

FAS; ARS Administrator B. T. Shaw; and Dr. F. E. Engleman, American Association of School Administrators.

"The membership voted to continue the ad hoc committee on public relations in agriculture to study further the development of a public relations program for ARI consideration at the next meeting.

"The report of the Conference Committee included a proposal of the California Cattlemen's Association for a national conference to discuss research on factors affecting efficiency of beef production and utilization in relation to U. S. grading system. It was recommended that next year's committee consider such a conference for 1959–60.

"Officers elected for the coming year are: President, Burt Johnson, National Cotton Council; Vice-President, C. H. Mahoney, National Canners Association; Secretary, M. E. Gillis, International Minerals and Chemicals Corporation."

W. M. Beeson presented a short review of his impressions of the meetings of the European Association for Animal Production held in Brussels in 1958. The Society's representative with the European Association, N. R. Joshi, presented the following report in absentia:

"The Fourth General Assembly and the Eighth Study Meetings of The European Association for Animal Production were held in Brussels from 23 to 27 July 1958.

"Seventeen Member countries and their organizations sent delegations to the meetings. There were also observers from two overseas countries. Besides these, there were observers from other international organizations. Dr. W. M. Beson, President of the American Society of Animal Production was at the meetings. Altogether over 90 livestock scientists participated.

"Two days were devoted to the presentation and discussions of seven papers dealing with various aspects of 'Problems of Productivity in Animal Production'.

"These papers were:

"1. The Influence of the Breeder on Progress in the Field of Animal Production.

"2. Present and Future Importance of Animal Production in Small Holdings in Lowland Areas.

"3. Present and Future Importance of Animal Production in Small Holdings in Mountain Areas.

"4. Influence of Recent Developments in Feeding, Breeding and Managment Techniques on Optimum Productivity in Horse Production.

"5. Influence of Recent Developments in Feeding, Breeding and Managment Techniques on Optimum Productivity in Cattle Production.

"6. Influence of Recent Developments in Feeding, Breeding and Managment Techniques on Optimum Productivity in Sheep and Goat Production.

"7. Influence of Recent Developments in Feeding, Breeding and Managment Techniques on Optimum Productivity in Pig Production.

"Meetings of the Study Commission on (1) Genetics, (2) Animal Feeding, (3) Horse Production, (4) Cattle Production, (5) Sheep and Goat Production and (6) Pig Production, were held concurrently.

"At the Study Commission on Genetics, two papers were presented by Professor H. L. LeRoy, Zürich, on:

"(1) Genetic and Environmental Variance and Genetic and Environmental Correlation between Individuals.

"(2) The Analysis of Variance and Regression as a Means of Estimating Heritability.

"At the Study Commission on Animal Breeding several papers were presented on the Measurement of Feed Values and Feeding Standards for European Livestock. The Study Commission is continuing its studies on the feed requirements and it is anticipated that this work will continue for the next two or three years.

"Also discussed at this meeting were problems of feed additives, mixed with intention or accidentally, and it was decided that they should be thoroughly investigated.

"At the Study Commission on Horse Production a report on 'The Present Situation and Future Trends of Horse Breeding in Europe' was presented. Although in various countries the horse numbers are decreasing on account of mechanization, the quality of the horse is not in any way affected. A great deal of attention is being paid to the breeding of sport horses.

"At the Commission on Cattle Production papers were presented on 'Progeny Testing in Beef Cattle Breeds' and with 'Evaluation of Carcasses'. A report on 'Inquiry on Herd-Book Keeping in Europe' was also presented.

"At the Commission of Sheep and Goat Production a paper on 'The Present Position of Goats in Europe' was presented. Also a paper on 'The Progeny Testing of Hams of Dairy Breeds of Sheep' was given by Dr. Finci.

"Information was supplied concerning the Meeting to be held in Rome in November 1958 on 'The Situation and Trends in the Sheep Industry of Europe with Special Emphasis on the Mediterranean Region'.

"At the Study Commission on Pig Production studies on artificial insemination in pigs attracted attention. A symposium on Artificial Insemination in Pigs is planned to be held in France during the spring of 1959. Two reports were presented on the influence of feeding and body composition of the pig."

W. B. Anthony presented the report of the Pasture and Forage Crop Committee:

"The fifteenth annual meeting of the Southern Pasture and Forage Crop Improvement Conference was held June 4–6, 1958, on the campus of Clemson Agricultural College, Clemson, South Carolina. Seventeen states and Puerto Rico were represented. One hundred and thirty-four people registered. As has been the practice for several years, the program followed the line of work being conducted in Regional Project S-12. Major areas of interest are plant breeding, environmental factors and cultural practices affecting forage crops and forage crop evaluation techniques. Continued interest has been shown in developing and improving methods for measuring quality and nutritive value of forage crops. An active animal research group regularly attends the annual meeting.

"Mr. Paul Henson, who has served as permanent secretary of the organization during the past ten years, resigned this year. Dr. D. E. McCloud of the Plant Industry Station, ARS, Beltsville, Maryland, was elected to fill the office.

"Dr. E. C. Holt, Plant Breeder, A & M College of Texas, is chairman of the Conference. The 1959 meeting will be held at Mississippi State College. The program is presently being developed."

J. K. Loosli presented the report of the Committee on Time and Place of Annual Meetings. His report was as follows:

"The Secretary of the Society mailed the following questionnaire to the membership:

Choice			
1st	2nd	3rd	Select and check only one proposition for first choice, only one for second choice, and only one for third choice.
( )	( )	( )	1. Hold the Annual Meeting in Chicago after Thanksgiving as at present.
( )	( )	( )	2. Hold a 2½ day meeting in Chicago starting Thursday noon, Thanksgiving Day and extending through Friday and Saturday.

Choice 1st	2nd	3rd	Select and check only one proposition for first choice, only one for second choice, and only one for third choice.
( )	( )	( )	3. Hold a three-day meeting between December 26th and the opening of college classes in early January and rotate the meeting among various cities or college campuses with adequate facilities. (A.A.A.S. always meets at this time).
( )	( )	( )	4. Meet during Wednesday, Thursday and Friday of the International Livestock Show Week.
( )	( )	( )	5. Meet for three days during the summer at various cities or at college campuses as Dairy Science and Poultry Science do now. (June seems undesirable because many of our members attend Dairy Science that month).
( )	( )	( )	6. Meet in Chicago on alternate years and at another location in interim years.

"A total of 1100 ballots were returned from a membership of 1829. The percentage distribution of the choices for the six proposals is summarized in table 1.

TABLE 1. PERCENTAGE DISTRIBUTION OF FIRST CHOICE VOTES AMONG PROPOSALS BY REGIONS

Region	Proposal on meeting place and time 1	2	3	4	5	6	% of total first votes	Chicago 1, 2, 4 and ½% of 6	Summer 5 and ½% of 6
Canada and foreign	7	10	10	10	50	13	2	33.5	56.5
East	25	4	5	23	36	7	15	55.5	39.5
Midwest	38	2	7	24	23	6	37	67	26
South	26	7	5	18	36	8	19	55	40
Southwest	21	3	2	13	48	13	11	43.5	54.5
West	9	12	5	9	54	11	12	35.5	59.5
No postmark	27	7	8	22	24	12	4	62	30
Total first Choices	27	3	7	20	35	8	100	54	39
All regions for choices									
Second	16	10	15	20	16	23	..	57	27
Third	18	9	11	17	13	32	..	60	29

Winter 1, 2, 3, 4, and ½ of 6=61%.
Summer 5 and ½ of 6=39%.

"It is seen that of the first choices 35% selected proposal 5, with 27 and 20% for No's. 1 and 4, respectively. If the first choices selecting Chicago as a place for the meeting are summed–54% favored Chicago vs. 39% to hold a summer meeting. Proposal 6 was selected most frequently as second and third choices.

"The voting was clearly inconclusive and the committee did not reach an unanimous agreement with regards to the wishes of the membership. Two propositions seemed to be favored: (1) to keep the meeting in Chicago, preferably at the present time or (2) to plan a summer meeting. The former seemed to be favored. This view was submitted to the Executive Committee with the suggestion that a revote should be taken with fewer proposals so that a clear decision could be reached."

W. E. Garrigus moved acceptance of the report. Charles Jordan seconded it. The motion was carried.

P. G. Woolfolk gave the report of the Pasture and Forage Committee as follows:

"The committee assisted in arranging a Joint Forage Evaluation Symposium with the American Society of Agronomy, American Society of Range Management, and American Dairy Science Association held at Purdue University, August 4, 1958, during the American Society of Agronomy annual meeting. Ten members of the American Society of Animal Production either gave or were listed as junior authors of papers at the Symposium.

"The committee represented the Society at a joint committee meeting concerning revision of the Manual of Pasture and Range Research Techniques also held on August 4, 1958. The decision was made to revise the manual with more complete treatment given to many of the topics included. Tentative assignments for the various organization involved were made.

"An Inter-Society Conference on 'Censusing Procedures for Reporting Production of Grazing Lands of the United States' was held on August 7, 1958. L. E. Harris and G. O. Mott represented the Society at this conference.

"It is recommended that the Society continue cooperation with the other societies involved in these matters."

L. E. Hanson gave the following report on the Ralston-Purina Research Fellowship Awards Committee:

"The Ralston-Purina Research Fellowship Awards Committee met in St. Louis, March 24, 1958. The Awards Committee was composed of five persons: James McGinnis, Washington State College, representing the Poultry Science Association; J. B. Frye, Louisiana State University, representing the Dairy Science Association; Robert MacVicar, Oklahoma State University, representing the American Association of Land Grant Colleges and State Universities; L. C. Ferguson, Michigan State University, substituted for C. A. Brandly, representing the American Veterinary Medical Association; and L. E. Hanson, University of Minnesota, representing the American Society of Animal Production.

"A total of sixty applications were reviewed by the committee. The average apparent quality of the applicants was of a very high order with many near perfect scholastic records. Awards were made on the basis of scholarship, undergraduate program, proposed research, recommendations and other pertinent data. Three awards were made in each of the following fields: Animal Husbandry, Dairy Husbandry and Poultry Husbandry. One award was made in Veterinary Medicine. The names of the awardees were published previously in the May issue of the Journal, page 489.

"The Ralston-Purina Company is to be commended for sponsoring these fellowships."

Report of the Tellers was read by W. M. Beeson. J. K. Loosli was elected Vice-President of the Society for 1959.

The Business Meeting was adjourned by W. M. Beeson at 9:35 p.m.

HONOR GUEST

1958

A. E. Darlow was born on a farm near Stillwater, April 24, 1899. He received his B.S. degree from Oklahoma State University in 1919, his M.S. degree from the same university in 1922 and his Ph.D. degree from the University of Wisconsin in 1939. His academic honors include membership in Phi Kappa Phi, Sigma Xi, Alpha Zeta and Phi Sigma.

From 1919 to 1935, Dr. Darlow was on the staff of the Animal Husbandry Department at Oklahoma State University where he advanced in professional rank from instructor to professor. He was Professor of Animal Husbandry at the University of Wisconsin from 1935 to 1943 and appointed Head of that Department in 1938. He returned to Oklahoma State University where he was Professor and Head of the Animal Husbandry Department from 1943 to 1953. In 1953, Dr. Darlow was appointed Dean of the College of Agriculture and Vice-President for Agriculture Sciences. From July 1, 1945 to January 1, 1946 he served as chairman of livestock program, U. S. Army, GI University, Shrivenham, England. In 1952, he served for a time as livestock program advisor, Point Four Program, Ethiopia. He was decorated Commander in Order of Star of Ethiopia in 1954. Dr. Darlow has served as Vice-President of the American Society of Animal Production (1953) and President (1954).

As head of the Animal Husbandry Department at Oklahoma State University, Dr. Darlow directed an extensive program of teaching and research. The latter included tests of management methods for cattle on Oklahoma grass, use of Oklahoma-grown grain for livestock, use of urea and other new products for livestock feeding, and the improvement of livestock through new breeding systems.

During his 10 years as coach of the livestock judging teams, 1925 to 1934, his teams won four 1st places, two 2nds and one 3rd place at the International Livestock Exposition and one 1st place, two 2nds and two 3rds at the American Royal. Dr. Darlow is known internationally as a judge of livestock having participated in judging such shows as the International Livestock Exposition, Chicago; English Royal (1957); Perth Shorthorn Show, Perth, Scotland (1946); Quayaquil Fair, Ecuador (1958); American Royal Livestock Show, Kansas City; National Western Stock Show, Denver, and other shows throughout the United States.

In addition to active membership in several organizations, Dr. Darlow, at present, is a member of the board of directors, National Stock Yards Company, Oklahoma City; member of executive committee, Oklahoma Medical Research Foundation; member of agriculture committee, United States Chamber of Commerce and director in Oklahoma Chamber of Commerce.

Dr. Darlow's home, 2004 West Admiral Road, is the gathering place for large groups of friends among the faculty and students and visitors to the university. Mrs. Darlow is the former Clara Priest. The Darlows have two children, Stella (Mrs. R. A. Shapiro) and Kathleen, a student at Oklahoma State University.

A. E. Darlow

Reproduction of a portrait presented to the Saddle and Sirloin Club in Chicago by the American Society of Animal Production on November 30, 1958. Dr. Darlow was the Honor Guest of the Society that year during its annual meeting.

AMERICAN FEED MANUFACTURERS' ASSOCIATION AWARD

1958

Orville George Bentley was born on March 6, 1918, and was reared on a ranch near Midland, South Dakota. He received a B.S. degree in Agriculture with a major in Chemistry in 1942 from the South Dakota State College. Following active service with the U.S. Army, he earned the M.S. degree in 1947 and the Ph.D. degree in 1950 in the fields of biochemistry and nutrition at the University of Wisconsin. Upon completion of his graduate work, he accepted a position as Assistant Professor in the Department of Animal Science at the Ohio Agricultural Experiment Station. He was promoted to the rank of Associate Professor in 1953 and to Professor in 1957. On October 1, 1958, taking a turn in a singular and productive career, he returned to the South Dakota State College as Dean of Agriculture and Director of the Agricultural Experiment Station.

Dr. Bentley is a scientist who has been motivated by a search for the basic understanding of animal nutrition. Much of his work has been of a fundamental nature; yet an underlying objective of his research has been the promotion of more efficient utilization of feeds and forages by cattle and sheep.

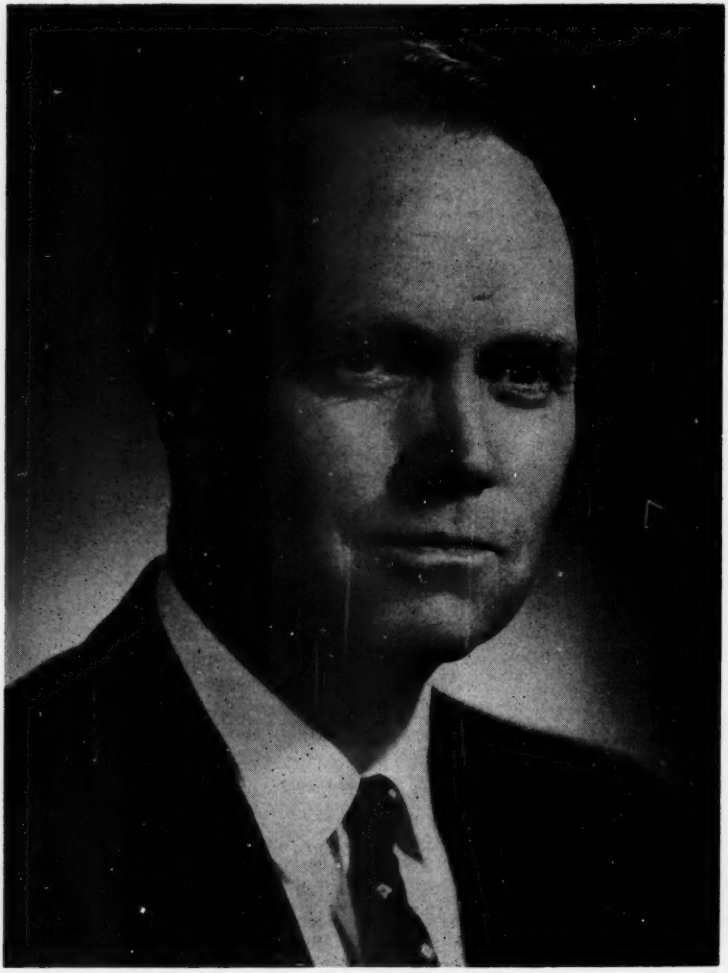
The contributions of Dr. Bentley as 1958 winner of the American Feed Manufacturers' Association Award impinge upon the complex problems of the digestion of feedstuffs by the microorganisms in ruminant animals. He has studied, with success, the *in vitro* fermentations of microorganisms of the rumen, and thereby further developing the so-called "artificial rumen" as an experimental technique. He experimentally verified the implication of *in vitro* studies that trace minerals, particularly cobalt, were limiting factors in the utilization of poor quality roughages by ruminants. He was among the first to study the nutritive value of dietary acetate, propionate, and lactate. He actively investigated the synthesis of vitamin B_{12} and the utilization of non-protein nitrogen by the rumen microflora.

Perhaps his most far-reaching contribution is the discovery that certain short chain fatty acids, certain amino acids, and biotin and para amino-benzoic acid stimulated the growth of cellulolytic organisms *in vitro*. With the developing concept that these stimulatory substances are derived from constituents of a good balanced ration, such findings will certainly form the basic principles for the more efficient formulation of rations for ruminants in the future.

It is a genuine and sincere pleasure to present to the Society the recipient of the 1958 American Feed Manufacturers' Association Award for original research in livestock nutrition, Dr. Orville G. Bentley.

Orville G. Bentley

O. G. Bentley received the 11th American Feed Manufacturers' Association Award, which was presented during the 50th Annual Meeting of the American Society of Animal Production in Chicago on November 28, 1958.



John K. Loosli

John K. Loosli received the 13th Morrison Award which was presented at the 50th Annual Meeting of the American Society of Animal Production on November 28, 1958.

THE MORRISON AWARD

1958

John Kaspar Loosli was born at Clarkston, Utah, May 16, 1909. He received his Bachelor of Science degree from Utah State College in 1931 and his Master of Science degree from Colorado A and M College in 1932. He completed the Doctor of Philosophy degree in Animal Nutrition at Cornell University in 1938.

During the past 24 years he has held the following positions: Instructor in Agriculture, Utah State Agricultural College, 1933–35; Assistant in Animal Nutrition, Cornell University 1935–38; Bureau of Biological Survey, 1938–39; Assistant Professor of Animal Nutrition, Cornell University, 1939–43; Associate Professor of Animal Nutrition, Cornell University, 1943–45; Professor of Animal Nutrition, Cornell University, 1945 to date.

Dr. Loosli is a member of Society for Experimental Biology and Medicine, American Institute of Nutrition, American Chemical Society, American Association for the Advancement of Science, American Dairy Science Association and American Society of Animal Production. In addition, he is a member of Sigma Xi, Phi Kappa Phi, Alpha Zeta and Gamma Alpha. From 1955 to 1958, he served as editor of the official publication of the American Society of Animal Production, Journal of Animal Science.

He is a member of the National Research Council's Committee on Animal Nutrition and has served as a member of several of its sub-committees, viz., The Nutrient Allowance of Fur Bearing Animals and Committee on Nutrient Allowances of Dairy Cattle, Chairman. Aside from many university committee assignments he is currently the graduate school representative from the field of Animal Nutrition, Cornell University.

Since the entrance of Dr. Loosli into the field of nutrition research in 1939, he has been the author or co-author of over 110 full-length publications which have appeared in recognized scientific journals. In addition to these, he has contributed over 49 other publications to the popular, or semi-scientific press on matters relating to his nutrition research. The subjects cover many species of animals including mink, foxes, laboratory rats, swine, sheep and dairy cattle. The critical agricultural problems to which his efforts have been directed include such ones as:

1. Importance of fat in the diets of lactating cows, rats and dairy calves.
2. Place of urea in ruminant feeding.
3. Importance of certain minerals such as calcium, phosphorus and cobalt in livestock nutrition.
4. Role of the fat-soluble vitamin A, D and E in the metabolism of sheep, lactating cows, swine and mink.
5. Economy of animal agriculture in the production of human food.
6. Importance of the rumen as a site of amino acid and vitamin synthesis.

7. Effect of antibiotics on the growth of dairy calves.
8. Relation of the stage of maturity of forage and its subsequent feeding value.
9. Amino acid requirements of swine and the development of techniques for their estimation.
10. Evaluation of by-product feeds for milk production.
11. Influence of diet on rumen development and function.

In 1956, Dr. Loosli revised the well-known book, "Animal Nutrition", by L. A. Maynard and is now a joint author. His interest has led him to investigate many problems of critical importance to the American farmer. Some of the more important contributions of Dr. Loosli may be classed in these categories:

1. Clear experimental evidence that rumen bacteria synthesize all of the essential amino acids.
2. Establishment of the amino acid requirements of swine for growth.
3. The value of antibiotics for dairy calves.
4 The role of vitamin E in the prevention of stiff lamb disease.
5. The value of certain protein feeds for milking cows.

While much of Dr. Loosli research has been concerned with fundamental problems of nutrition and metabolism, he has the rare gift of extrapolating the results towards solution of practical problems. He has continually examined the need for research in areas of immediate concern to the farmer, especially in attempting to develop recommendations concerning new products that are being presented to the farmer for consideration. His research on vitamins for calves, antibiotics for calves and tocopherols for milk production are cases in point. Each of these contributions has resulted in substantial savings to the farmer.

In recognition of his outstanding research, Dr. Loosli received the American Feed Manufacturers Award from the American Society of Animal Production in 1950 and the Borden Award in Dairy Production of the American Dairy Science Association in 1951.

Apart from his contribution in research results, Dr. Loosli can be classed as a master at directing or leading graduate students. He has been the committee chairman of 26 graduate students and served as a minor committee member for even more. His friendly, quizzical, encouraging approach to the new or confused prospective researcher has resulted in the molding of many successful nutrition scientists. His major students may now be found as heads of departments, commercial feed company nutritionists, as well as successful nutrition scientists in over 11 universities.

Truly in the short space of 19 years, Dr. Loosli has contributed much to the field of agriculture both directly from his own hand and indirectly from the expanding work of his students. In him we find a rare combination of a fine practical knowledge of animal husbandry with a thorough understanding of the underlying sciences and their utilization in advancing the field through teaching and research.

In recognition of distinguished service over a period of 25 years or more, the title of

Fellow

of the

American Society of Animal Production

has been conferred upon the following men:

Earle W. Crampton. Dr. Crampton was born in Middletown, Connecticut. He received his B.S. degree from the Connecticut Agricultural College, his M.S. degree from Iowa State College and his Ph.D. degree in Nutrition from Cornell. He was lecturer in Animal Husbandry at MacDonald College in 1922, made Associate Professor in Animal Nutrition in 1933, and Chairman of the Department of Nutrition in 1947. He is a member of the Canadian Council of Nutrition; Fellow, Royal Society of Canada; Fellow, Agricultural Institute of Canada; Commander, Order of Agricultural merit. He received the American Feed Manufacturers' Association Award in 1948, the Morrison Award in 1955, was Vice-President of the American Society of Animal Production in 1950 and President in 1951. He is the author of a text book on animal nutrition and has published extensively in that field.

Carl F. Huffman. Dr. Huffman was born in Tonganoxie, Kansas. He received his B.S. degree from Kansas State College in 1917 and his M.S. degree from Minnesota in 1922. He was a scholar in Wisconsin in 1929 and received his Ph.D. degree from that institution in 1933. He was appointed as instructor of dairying at Kansas State College in 1919, Assistant at Minnesota in 1921 and at Michigan State in 1922. He was made Research Professor at Michigan State in 1947. He was the Borden Award winner in 1937 and received the American Feed Manufacturers' Association Award in 1954. He has carried on an extensive program of teaching and research in the field of general ruminant nutrition. His specialties are dairy cattle production and nutrition.

Henry H. Kildee. Dr. Kildee was born in Osage, Iowa. He received his B.S. and M.S. degrees from Iowa State College. He was made an Honorary Doctor in Agriculture at the North Dakota Agricultural College in 1940. He became an instructor in Animal Husbandry at Iowa State College in 1909 and Professor of Animal Husbandry and Chief of Dairy Husbandry in 1915. He became Chief of the Division of Dairy Husbandry at Minnesota in 1916 and returned to Iowa State College as Head of Animal Husbandry in 1918. He was appointed Vice-Dean of Agriculture in 1923 and Dean in 1933. He is President of the International Livestock Exposition and a member of the Board of Governors of the American

7. Effect of antibiotics on the growth of dairy calves.
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Truly in the short space of 19 years, Dr. Loosli has contributed much to the field of agriculture both directly from his own hand and indirectly from the expanding work of his students. In him we find a rare combination of a fine practical knowledge of animal husbandry with a thorough understanding of the underlying sciences and their utilization in advancing the field through teaching and research.

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Royal. He is a member of the National Brucellosis Committee and has received gold medals and distinguished service awards from the American Farm Bureau Federation and Costa Rica. He has judged leading shows of the United States as well as some in foreign countries. He was Vice-President of the American Society of Animal Production in 1934 and President in 1935. His interests are livestock production for meat and milk, and conservation of land and water resources.

Jay L. Lush. Dr. Lush was born in Shambaugh, Iowa. He received his B.S. degree at Kansas State College in 1916 and his M.S. degree from the same institution in 1918. He received his Ph.D. degree in Genetics from Wisconsin in 1922. He served on the staff at the University of Wisconsin and at Texas A and M College. He is currently Professor of Animal Breeding at Iowa State College. He received the Morrison Award in 1946. He was Secretary-Treasurer of the American Society of Animal Production from 1935–37, Vice-President in 1938 and President in 1939. He has written several text books on animal breeding and statistical genetics. In 1958, he was awarded the honorary degree, Doctor of Agriculture, by the Royal Veterinary and Agricultural College of Denmark.

Hugh C. McPhee. Dr. McPhee was born at Hastings, Maine. He received his B.S. degree from Maine and his M.S. and D.Sc. degrees from Harvard. He was scientific aid at the Maine Experiment Station and joined the Bureau of Animal Industry, U.S.D.A., in 1923. He was appointed Animal Husbandman and later Senior Animal Husbandman in that Bureau. He became Chief of the Animal Husbandry Division in 1935 and Assistant Chief of the Bureau in 1942. With the reorganization of the Department of Agriculture, he was made Assistant Director of Livestock Research, which position he held until his retirement. He has represented the United States in various foreign conferences and congresses. He has written many publications. His specialty is animal breeding and genetics.

Roscoe C. Pollock. Dr. Pollock was born at Martinsburg, Iowa. He received his B.S. and M.S. degrees and an honorary Ph.D. degree from Iowa State College. He has served as county agent at Farmington, Minnesota, as assistant county agent leader in South Dakota and as county agent leader in North Dakota. He served as director of extension of the Holstein-Friesian Association and joined the National Live Stock and Meat Board as Secretary and General Manager when it was organized in 1922. During World War II, he was a member of the National Research Council Quartermaster Research and Development Committee. He also served on the National Protein Committee, U.S.D.A. and on the Agricultural and Industrial Committee of the Northern and Eastern Regional Research Laboratory. He has rendered service to the American Society in initiating and supporting the meat sessions and also in providing publicity service for the annual meetings.

Joe M. Vial. Mr. Vial graduated from the University of Illinois. He farmed for a time near Cherokee, Iowa, and was active in organizing the Iowa Farm Bureau Federation. He assisted in the formulation of livestock extension programs for the states of Iowa, Illinois, Pennsylvania, Maryland, New York and Maine. He was an exhibitor and importer of Percheron horses and served as a national director of the Percheron Association. He is widely sought as a judge of state and national shows. He is interested in youth programs such as the 4-H and FFA. He has served as Secretary of Beef Cattle Breed Associations in Maryland, and as Secretary of the Eastern National Livestock Show from 1947–54. He has developed marketing systems for feeder cattle and also for pigs and lamb pools.

Edward N. Wentworth. Dr. Wentworth was born at Dover, New Hampshire. He received his B.S. and M.S. degrees and an Honorary Doctor of Agriculture degree from Iowa State College. He also attended Cornell University, Harvard and Kansas State. He served on the staff of Iowa State College, Chicago Veterinary College and Kansas State College. From 1922–54 he served as director of the Armour Live Stock Bureau. He was Associate Editor of the Breeder's Gazette. During World War I he served as Captain of Field Artillery in the United States Army. His interests are in genetics, animal breeding and economics. He is a delightful writer and as author and co-author with his brother-in-law, Charley Towne, he has greatly enriched animal husbandry literature.

Selection of these Fellows was announced at the 50th Anniversary meeting of the Society in Chicago, November 28, 1958.

MEMORIALS

F. W. Atkeson

F. W. Atkeson, 64, Head of the Department of Dairy Husbandry, Kansas State College, Manhattan, died April 4, 1958, at Riley County Hospital. Mr. Atkeson was injured in an automobile accident in September of 1957 and had been in failing health since. He had not been in his office since early February.

A native of Butler, Mo., he received his B.S. in Agriculture from the University of Missouri in 1918 and his M.S. from Kansas State College in 1929. He was an assistant in the Kansas State Dairy Department from 1918 to 1920, a field representative for the American Jersey Cattle Club from 1920 to 1921, and Professor of Dairy Husbandry and Head of the Department at the University of Idaho, Moscow, from 1921 to 1935. In 1935 he returned to Kansas State College as Head of the Dairy Department.

Mr. Atkeson was honored recently by friends in the feed industry, who established the Atkeson Loan Fund to recognize his work as chairman of the Kansas Formula Feed Conference for many years. He was also cited earlier this year by the Kansas Inter-Breed Dairy Cattle Council as "Kansas Dairy Leader". He became the sixth Kansan to be so honored.

Mr. Atkeson judged many of the nation's top dairy shows and had been an official classifier for three dairy breeds. He was author or co-author of more than 100 publications dealing with dairying. He was a member of the American Society of Animal Production, American Dairy Science Association and American Genetics Association.

Survivors include his widow, Ferol; a son, George, U. S. Air Force, Alaska; two brothers, Ralph Wendell, Jefferson City, Missouri, and Clarence E., Wichita, Kansas, and a sister, Mrs. J. W. McCreery, Savannah, Georgia.

Phillip Martin Brandt

Phillip Martin Brandt, former Professor of Dairy Husbandry and Head of the Department of Dairying at Oregon State College until his retirement on June 30, 1957, passed away on October 3, 1958, at the age of 70 years. Mr. Brandt had been in poor health during the year prior to his death.

Professor Brandt was born on August 6, 1888, at Nevada, Missouri, and was reared in Missouri. He received his B.S. in Agriculture in 1910 and his A.M. degree in 1913 from the University of Missouri. He held the position of Secretary of the Missouri State Dairymen's Association from 1912–14. While working for his A.M. degree, he was an Instructor in Dairy Husbandry and was promoted to Assistant Professor of Dairy Husbandry in 1913; then to Assistant to Dean and Director and Superintendent of Short Courses at the University of Missouri.

In 1917, Professor Brandt came to Oregon State College where he served on the staff for 40 years. His record is outstanding both in the advancement of the dairy industry as well as in civic affairs. Mr. Brandt served as President of the original Oregon Dairy Council 1924–33, elected Mayor of Corvallis 1933–34, and served diligently on various state committees. He served as President of the Corvallis Chapter of the Oregon State Employees Association and as the Director of Retirement and also Vice-President of the State Association. He was assigned to numerous committees for the American Dairy Science Association and the Western Division of the American Dairy Science Association during his tenure at O.S.C. Among Mr. Brandt's better known publications were policy statements prepared for special occasions, "The Dairy Industry at the Crossroads", January 17, 1945, and "Guide Posts for the Dairy Industry", January 8, 1954.

Professor Brandt fostered research related to both dairy production and dairy processing. Studies included brucellosis control, butter marketing, milk production

costs, artificial breeding of cattle, bovine reproduction with low-carotene rations, relation of milk quality to equipment, buildings and production methods, irrigated pastures, ensiling methods, improvement in manufacturing, processing and packaging of butter, cheese and ice cream, and research dealing with the normal composition and re-evaluation of cows milk.

Profesor Brandt was a member of Gamma Sigma Delta, Sigma Xi, American Dairy Science Association, American Society of Animal Production, Masonic fraternities and Sons of the American Revolution.

Mr. Brandt is survived by his wife, Mattie May Torr Brandt, a son, Phillip M. Brandt, Jr., Salem, Oregon, a daughter, Mrs. Harriett Anne Hutchenson, Northridge, California, and five grandchildren.

Edmund W. Cheng

Edmund W. Cheng, Assistant Professor of Animal Husbandry at Iowa State College, died near Iowa City on October 6, 1958, as the result of an automobile accident. Cheng was born in Tsungkao, China, on November 24, 1916. He received his B.S. degree in Biology from Southwest Associated University in China in 1940. Also while in China he received the M.S. degree in Physiology from Tsing Hua University in 1943. On July 17, 1940, he was married to Dorothy Wei Cheng who survives him. His mother and four brothers also survive him.

Edmund, or "Ed", as he was familiarly known to his friends, came to Iowa State College in 1946 as a graduate student in Animal Husbandry where he earned the M.S. degree in 1948 and the Ph.D. degree in Animal Nutrition in 1952. He joined the Animal Husbandry staff as a research associate in Ruminant Nutrition in 1951 and advanced to the rank of Assistant Professor in 1956. He was the author of more than 30 scientific papers, the majority of which dealt with rumen physiology and the nutritional significance of estrogens in fattening cattle and in lamb production.

Cheng was a member of the Presbyterian Church in Ames, Iowa. He was a member of a number of honorary and professional societies, including Sigma Xi, Phi Kappa Phi, Phi Lambda Upsilon, American Society of Animal Production, American Dairy Science Association, American Chemical Society, Iowa Academy of Science, New York Academy of Science and the Society for Experimental Biology and Medicine.

His ability as a scientist in the laboratory and his friendly desire to assist graduate students and fellow researchers made him a real champion to those he contacted. His untimely death marks the end of an outstanding scientific career and a real loss to those engaged in ruminant nutrition.

Nicholas Rood Ittner

Nicholas Rood Ittner, Specialist in Animal Husbandry at the Meloland Experiment Station of the University of California, passed away unexpectedly February 1, 1958. He was born in Mt. Vernon, Iowa, in 1911 and moved to California when a child. He was graduated from the University of California at Davis in 1934. Following graduation he was Swine Herdsman at the great Fontana Farms Company, California, and the University of California for four years. He then assumed his research position with the latter Institution which he held until his passing. From 1942 until 1946 he served as an officer in the United States Army and served overseas in the North African campaign and Normandy landing. Upon his return to work in 1946 he was assigned to the Meloland Experiment Station in Imperial Valley. His research record was outstanding as testified by a list of more than 45 research publications as author and co-author over the brief years he worked. His research programs were carefully planned and meticulously executed. His early studies were cooperative with the late Dr. Elmer Hughes on swine nutrition, and more recently he has cooperated with the Department of Agricultural Engineering and Agricultural Research Service, U.S.D.A.,

in a series of very fruitful studies concerning livestock adaptation to hot climates. Two of these joint papers were selected as being among the five outstanding papers in Agricultural Engineering for the years 1950 and 1954 and a third, the most outstanding, for 1955.

Nick will be remembered for his devotion to his research and his pleasant, happy personality. Though at a considerable distance from his colleagues, he kept in close contact and was extremely interested in the American Society of Animal Production. He was a regular contributor to the Journal of Animal Science.

He is survived by his wife, Elsa, whom he married in 1942, and who has returned to Davis to reside.

Reuben F. Johnson

Reuben F. Johnson, Superintendent of the University of Idaho Branch Agricultural Experiment Station at Caldwell, died March 13, 1958. "Rube", as he was known to his countless friends, was born in Idaho Falls in 1899. He graduated from the University of Idaho's College of Agriculture in 1921 with a major in Animal Husbandry.

He joined the Agricultural Experiment Station staff in 1925 and was assigned to feeding studies at Caldwell Branch Station. This was at a time when little steer or lamb feeding was done in Idaho and feeder steers and lambs were shipped out by the trainload right beside trainloads of hay.

Johnson became Superintendent of the station in 1932. With the firm belief that both feeder stock and hay should be kept at home, he directed the Caldwell steer and lamb feeding research to develop rations and methods that would make full use of Idaho-grown feeds.

He was author or co-author of 40 publications of work conducted at the Caldwell Branch Station. He was a charter member of the Idaho Chapter of Alpha Zeta and an active member of Sigma Xi.

He is survived by his wife, Christine; a son, Reuben Jr.; and two daughters, Marjorie and Helen.

Frank B. Morrison

Frank B. Morrison, Emeritus Professor of Animal Nutrition and Animal Husbandry, Cornell University, passed away suddenly on April 7, 1958 in Atlantic, Iowa. He and Mrs. Morrison were enroute to Clinton to visit their son, Spencer, and his family after participating in livestock meetings in the southwestern states. Since his retirement from active service at Cornell in 1955, Professor and Mrs. Morrison had spent the winters in Sarasota, Florida, and the summers at Upper Saranac Lake, New York.

Professor Morrison was born in Fort Atkinson, Wisconsin on May 19, 1887 and spent his boyhood on a farm near Marshfield. After graduation at the top of his class, he taught country school for three years in order to accumulate funds for further education at the University of Wisconsin.

Immediately upon graduation with highest honors from the University of Wisconsin, Professor Morrison became administrative assistant to Dean H. L. Russell and soon was appointed to the position of Instructor in Agricultural Chemistry. He was promoted rapidly to the rank of Professor and in 1919 was appointed Assistant Director of the Wisconsin Agricultural Experiment Station, a position he held until 1927. At this time Professor Morrison accepted a position as Director of the New York State Agricultural Experiment Station at Geneva and the Cornell University Agricultural Experiment Station at Ithaca, New York.

Professor Morrison became head of the Department of Animal Husbandry at Cornell University in 1928. He served in this capacity until 1945 when he asked to be relieved of his administrative duties as head of the Department in order to have more time for writing and the guidance of graduate students. He continued as a Professor until his retirement in 1955.

Great strides were made in Animal Husbandry at Cornell and in New York State during Professor Morrison's tenure as head of the Department. New livestock barns were built, better livestock were purchased, graduate training programs were strengthened, keen young scientists were added to the staff, undergraduate courses were revitalized, and extension programs were made more informative and valuable.

Professor Morrison traveled extensively at home and abroad. At various times he conducted livestock studies and surveys for the governments of the Philippines, Germany, Argentine and Venezuela. While he was probably best known throughout the world for his books, Feeds and Feeding and Feeds and Feeding, Abridged, Professor Morrison took his greatest pride in the accomplishments of his former students. Numbered among them are college and university presidents, deans of colleges of agriculture, outstanding scientists and teachers, heads of departments, leaders in industry and government, and successful breeders and farmers.

Professor and Mrs. Morrison established in 1946 an annual award, later made perpetual through a trust fund, which enables the American Society of Animal Production to give a substantial cash prize each year to a scientist selected by the Society for his outstanding research in livestock production. Also, in 1950, they established an endowed fellowship at Cornell University to foster the training of graduate students in the field of livestock feeding.

Possessed with a keen, analytical and brilliant mind and great energy and capacity for work, Professor Morrison was an inspiration to all who were fortunate enough to know him or to be associated with him. He had an unusual ability to translate fundamental research in chemistry and nutrition into the practical application of more efficient livestock feeding. No man contributed more to the dairy and livestock industries during the past 40 years than has Professor Morrison.

His integrity, ambition and generosity in all his work as a teacher, scientist, administrator and author earned for Professor Morrison a place of distinction and esteem throughout the world. He was truly one of the greatest leaders in agricultural science.

Professor Morrison was a member of Alpha Zeta, Sigma Xi, Phi Kappa Phi and Phi Lambda Upsilon. He received honorary Doctor of Science degrees from the University of Vermont in 1947 and the University of Wisconsin in 1950. He served as President of the American Society of Animal Production in 1920 and his portrait was hung in the Saddle and Sirloin Club in 1938.

Professor Morrison is survived by his wife, Elsie Bullard Morrison, who worked closely with him in his writing, and by two sons, Roger and Spencer, and nine grandchildren.

William E. Pugh

W. E. "Bill" Pugh, Extension Animal Husbandman at the University of Missouri, collapsed and died of a heart attack while judging 4-H Club livestock entries at the Howell County 'Heart of the Ozarks' Fair in West Plains, Mo., early in September. He was 42 years old.

The Missouri extension worker, widely known and respected by the farm people with whom he worked in his home state and other midwestern areas, had been University livestock specialist since joining the Extension Service state staff in 1951.

Earlier, from 1942 to 1951, he had been County Agent of Monroe County, Mo., with the exception of a three-year tour of duty with the Navy in World War II.

Pugh, a native of Mt. Vernon, Mo., graduated from the University of Missouri in 1937 with a B.S. degree in agriculture. Shortly after completing his college work, he accepted a job with the Farmers Home Administration in Fayette, Mo., and stayed at that job until he joined the Missouri Extension Service.

He is survived by his wife, Sue, and two children, Bob and Nancy, all of Columbia, Mo.

William Hugh Riddell

Dr. William Hugh Riddell, Chairman of the Department of Animal and Dairy Husbandry, University of Vermont, died June 27, 1958, at Burlington.

Born in Fort Langley, B.C., Canada, Dr. Riddell became a naturalized citizen of the United States in 1940. He received his B.S. degree from the University of British Columbia in 1922 and his M.S. and Ph.D. degrees from the University of Minnesota in 1924 and 1932, respectively.

During World War I, he served as a Private in the Canadian Army in England, 1917 to 1919. In World War II, he was nutrition officer at Walter Reed Hospital, Washington, D.C.; and later was the first postwar agricultural attache in The Netherlands, serving under Supreme Headquarters of the Allied Expeditionary Forces with the rank of Lieutenant Colonel. For his services, the Dutch government decorated him with the order of Orange-Nassau, rank of Commander.

Before coming to the University of Vermont, Dr. Riddell was engaged in teaching and research at the University of Minnesota and Kansas State College and was Head of the Dairy Science Department at the University of Arizona.

Dr. Riddell's teaching and research accomplishments have added significantly to the understanding of both the basic and applied fields of animal and dairy husbandry. His research publications on the vitamin content of milk, the mineral metabolism of cattle, and the nutritive value of pasture and forage crops are considered to be major scientific contributions to the field of animal science.

Dr. Riddell was a member of the American Dairy Science Association, the American Society of Animal Production, and Sigma Xi. In his community, he was active in the Rotary Club, the College Street Congregational Church, and the Burlington Community Y.M.C.A.

Dr. Riddell's passing will be felt deeply by all who knew and loved him. They will miss his friendliness, his understanding, and his sincere interest in the welfare of others. He will be remembered by his many friends in the dairy industry, with which he was closely associated, and his many former students to whom he contributed so much.

E. L. Shaw

E. L. Shaw, commonly known as Colonel Shaw, was born on November 4, 1877. He received his B.S. degree in Agriculture from Ohio State University in 1902 and served as an Assistant in Agriculture at the Missouri Experiment Station in 1903, and Assistant Professor of Agriculture in charge of Animal Husbandry at the New Hampshire Experiment Station from 1903 to 1907. He was in charge of the sheep and goat investigations for the United States Department of Agriculture from 1907 until 1913 and was in charge of the United States Experimental Farm at Beltsville, Maryland, from 1913 until 1917. During the period from 1917 to 1948, Colonel Shaw served on a half-time basis as a sheep specialist with the Agricultural Extension Service at West Virginia University. During this period of time, almost 31 years, Colonel Shaw operated a very fine farm in Ohio and was recognized as an outstanding breeder of Southdown sheep and one of the most highly recognized sheep judges in the country. Colonel Shaw died September 7, 1958.

Charles M. Thompson

Dr. Charles M. Thompson passed away Monday, April 7, 1958 at Beardstown, Illinois.

Dr. Thompson was a native of Kentucky. He received his B.S. degree in Agriculture from the University of Kentucky in June 1947 and his Master's degree in Agriculture from the University of Kentucky with a major in Animal Nutrition in March 1949. Dr. Thompson also held the Doctor's degree with a major in Animal Nutrition from

Oklahoma State. He returned to the University of Kentucky as Assistant Professor of Animal Nutrition in charge of the Animal Nutrition Section, on February 1, 1953.

On August 30, 1955, he resigned from the University of Kentucky to accept a position with the Colorado Milling Company of Beardstown, Illinois, where he served as Director of Research and Nutrition for approximately two years, resigning December 15, 1957 to enter the feed consulting business.

Survivors are his wife, Esther, and three children, Carl, Elizabeth Ann and Deborah Kay, as well as his parents, Mr. and Mrs. John F. Thompson of Stark, Kentucky, and three brothers living in Kentucky, and a sister in Florida.

Dr. Thompson was well known throughout the feed industry.

Elwin L. Willett

Dr. Elwin L. Willett, one of the most distinguished scientists at Michigan State University, member of the M.S.U. Dairy Department, passed away the evening of Wednesday, October 22, 1958.

Dr. Willett was an alumnus of Michigan State, graduating in 1937. His Master of Science degree was obtained from the University of Nebraska in 1938 and his Ph.D. from Cornell in 1941. He is well remembered by some of his older instructors at Michigan State as one of the most brilliant students to be graduated from the school.

Dr. Willett was a reproduction physiologist. He attained international prestige when he successfully transplanted the fertilized ovum from one dairy cow to another. He had been engaged in both bovine male and female reproduction physiology at his Alma Mater since July, 1957.

After receiving his degree, Dr. Willett did research work at both the University of Puerto Rico, the University of Hawaii, and later acted as director of research for the American Foundation of Biological Research in Madison, Wisconsin. He was a member of many scientific societies.

Dr. Willett is survived by his widow, a former home demonstration agent at Traverse City, and by four children.

As an individual, Dr. Willett was very quiet, unassuming, and a warm personal friend of everyone he knew. The Dairy Department personnel feel that they have lost one of their outstanding people.

Laurence Merriam Winters

Laurence M. Winters was born at Lake City, Minnesota. He earned his B.S. degree (in Animal Husbandry) in 1919 and his Ph.D. degree (in Zoology) in 1932, both at the University of Minnesota. His M.S. degree was earned at Iowa State College in 1920. His search for knowledge led him to study at the University of Wisconsin in 1925 and at the Boyce Thompson Institute (Harvard University) in 1927.

Dr. Winters was a Professor of Animal Husbandry at the University of Saskatchewan from 1920 to 1928. In the latter year he returned to Minnesota as Associate Professor in charge of Animal Breeding in the Animal Husbandry Department. Promotion to rank of Professor came in 1934. He retired from the University staff in 1956 to accept a post as an Agricultural Advisor to the Government of Iraq, where he acted for the International Cooperation Administration of the U. S. State Department. He was in Baghdad at the time of his death, March 16, 1958. He was a member of the American Society of Animal Production, American Genetics Association, Genetics Society of America, Society of the Sigma Xi, Phi Kappa Phi, and Gamma Sigma Delta.

Loving children, Laurence and Ellen Winters adopted one and took three others into their home.

Dr. Winters was a world leader in his chosen field, acclaimed as such by colleagues at Minnesota and throughout the United States and the rest of the world. The influence of his work and thought, worked through his numerous scientific and popular

publications, his many students, and his warm personal contacts with farmers in Minnesota and elsewhere, had an important impact on animal breeding practice. Perhaps his greatest contribution was his early insistence that animal breeding problems be analyzed in the light of the emerging facts of genetic science. He conducted pioneer researches on crossbreeding, performance testing, and breed formation. His work in the first of these areas culminated in almost universal adoption of crossbreeding for U. S. market hog production. The Minnesota No. 1 and No. 2 breeds of swine, developed by Dr. Winters, have achieved wide recognition. More important than these breeds, however, is the reminder that came with them that no area of human endeavor should be hampered by static thought patterns.

In recognition of his very real accomplishments the American Society of Animal Production in 1948 made Dr. Winters recipient of the Morrison Award for outstanding contribution to Animal Husbandry.

Laurence M. Winters earned an enduring place in the history of his profession. Besides relatives he leaves a multitude of admirers and warm personal friends.

CHAIRMEN OF COMMITTEES, PROGRAM CHAIRMEN AND SPECIAL ASSIGNMENTS FOR 1959

Standing Committees

Committee on Research ..... S. L. Hansard
Committee on Monograph ..... C. E. Terrill
Committee on Extension ..... M. D. Lacy
Committee on Membership ..... J. K. Loosli
Committee on Teaching ..... Roy Kottman

Special Committees

Committee on Information ..... Carl Neumann
Committee on Necrology ..... A. L. Anderson
Committee on Nominations ..... J. C. Miller
Committee on Morrison Award ..... J. K. Loosli
Committee on American Feed Manufacturers Award ..... G. K. Davis
Committee on Registration and Facilities ..... D. E. Becker
(Vice Chairman) ..... G. D. Pendergrass
Committee on Resolutions ..... L. E. Casida
Committee on Fellows ..... W. W. Green
Committee on Extension Award ..... W. M. Beeson

Program Committees

Breeding and Genetics ..... R. R. Shrode
Meats ..... J. C. Pierce
Nutrition ..... Damon Catron
Pastures and Forages ..... P. G. Woolfolk
Physiology ..... J. F. Lasley

Special Representatives

American Association for Advancement of Science Council .... T. C. Byerly
Committee on Intersociety Cooperation and
Scientific Manpower Commission ..... J. E. Foster
Division of Biology and Agriculture,
National Research Institute ..... C. F. Sierk
European Association for Animal Production ..... N. R. Joshi
Ralston Purina Award Committee ..... L. E. Hanson
Pasture and Forage Crop Improvement Committee ..... W. B. Anthony

NEWS AND NOTES

Ralston Purina Research Fellowship Awards Program. Again this year, ten Fellowships in the amount of $1800 each will be granted for graduate study in Agricultural Research. Awards will be made in the fields of (1) nutrition and physiology research as applied to dairy, poultry and animal husbandry, and (2) research in transmissible diseases of livestock and poultry.

Any individual qualified for graduate study in any Land-Grant Agricultural College or approved Veterinary College (including Canadian Colleges) who possesses desirable personal qualifications and submits a completed application may be eligible. The application and required information must be in the hands of the Awards Committee by March 1 of the year the Award is to be made. The selection of the recipients of the Annual Research Fellowship Awards, as well as the rules governing the awards, shall be made by a committee of an officially appointed representative of each of the following organizations: Poultry Science Association, American Veterinary Medical Association, American Dairy Science Association, American Society of Annual Production, American Association of Land Grant Colleges and State Universities and the Ralston Purina Company.

Application blanks for these Fellowship Awards may be obtained by writing the Ralston Purina Research Awards Committee, c/o Mr. J. D. Sykes, Ralston Purina Company, St. Louis 2, Missouri.

The Tenth International Congress of Genetics was held at McGill University, August 20–27, 1958, under the distinguished patronage of His Excellency, the Right Honourable Vincent Massey, C. H., Governor General of Canada. The Right Honourable John George Diefenbaker, Prime Minister of Canada, and other eminent statesmen and scholars of Canada, constituted the Honourable Committee. McGill University was the host institution of the Congress which was sponsored by the Genetics Society of America, and eleven other biological organizations of North America acted as co-sponsors. These co-sponsors were: The Genetics Society of Canada, the Agricultural Institute of Canada, the American Cancer Society, the American Eugenics Society, the American Genetic Association, The American Society of Agronomy, The American Society for Animal Production, The American Society for Horticultural Science, The American Society of Human Genetics, the American Society of Naturalists and the Society for the Study of Evolution.

The membership of 1672 greatly exceeded the 863 of the previous Congress, and consisted of representatives from fifty countries. There were 1310 Active Members (of whom 72 were non-participating, i.e. did not attend), 362 Associate Members (of whom 12 were non-participating) and 196 children. Twenty countries were represented by 58 official delegates who included representatives of 16 overseas universities.

The scientific programme consisted of 33 reports in seven Symposia, 543 Contributed Papers in 43 sessions, three Public Lectures, a session of 40 Demonstrations and a Panel Discussion on *The Teaching of Genetics*. It is obviously impossible to comment, even briefly, on the contents of these contributions. A special feature was an extensive Exhibit illustrating the topic *Genetics in the Service of Man,* covering the entire ice area (85′ x 200′) of the McGill Winter Stadium, and open to the public.

The Proceedings consist of *The Programme* (71 pages) and *Volume II: Abstracts* (339 pages, 576 abstracts) both of which were available at registration, and *Volume I* which will contain the full texts of the Presidential Address, Invitation Papers and Public Lecturers, and records of Business Meetings, etc., and will be published about April, 1959. These Proceedings can be ordered from the University of Toronto Press. Toronto, Ontario, Canada.

Purdue University announces the establishment of the *Population Genetics Institute* to coordinate an expanding research program in population genetics. The major objective of the Institute will be to investigate the effects of various types of gene action on response to selection under various mating systems and varying environmental conditions. In addition to theoretical studies, problems will be investigated experimentally with laboratory organisms such as Drosophila, Tribolium and mice.

Facilities for the Institute in Purdue's new Life Science Building will include offices, fully equipped laboratories, and three specially designed controlled environment chambers. The facilities of the statistical and computing laboratory, including a digital computer, will be used for expanding the theoretical or mathematical approach to problems of population genetics. Dr. A. E. Bell, professor of Poultry Science, was named chairman of the Institute. Others from the Purdue staff named to the Institute include Drs. V. L. Anderson, B. B. Bohren, S. C. King, W. H. Kyle, J. H. Martin, T. G. Martin and H. E. McKean.

The fourth biennial *Symposium on Animal Reproduction* will be held June 18, 19, and 20, 1959, at the University of Illinois, Urbana, Illinois. The theme of the program will be, "The Effect of Germ Cell Damage on Reproduction". Inquiries concerning program and reservations should be addressed to P. J. Dzuik, 1[illegible] Animal Genetics, University of Illinois, Urbana, Illinois.

Southern Regional Graduate Summer Session in Statistics. The 1959 session will be held at North Carolina State College, Raleigh, from June 8 to July 17. Like previous sessions under this program, it is intended to serve: (1) teachers of introductory statistical courses who want formal training in modern statistics; (2) research and professional workers who want intensive instruction in basic statistical concepts and modern statistical methodology; (3) professional statisticians who wish to keep informed about advanced specialized theory and methods; (4) prospective candidates for graduate degrees in statistics; and (5) graduate students in other fields who desire supporting work in statistics.

The National Science Foundation is offering grants to college teachers of introductory statistics who wish to attend the 1959 session. Stipends of $75 per week for the six weeks of the session plus $15 per week per dependent (up to four) will be made available for a maximum of 30 applicants; in addition, there will be a travel allowance of 4 cents per mile, round trip. Tuition and fees will be paid by the National Science Foundation. Participants will normally be enrolled in classes for graduate credit.

Applicants for National Science Foundation grants will be selected on the basis of interest in continued teaching of statistics, evidence of excellence as a teacher, previous academic record of the applicant, number of introductory statistics courses now teaching, and number of students contacted. Applications must be received not latter than February 16, 1959, to be assured of full consideration. Applicants will be notified of the selection committee's action not later than March 16, 1959, and must accept (or decline) a fellowship award not later than April 1, 1959.

Requests for application blanks for the summer school and for National Science Foundation grants should be addressed to: F. E. McVay, Department of Experimental Statistics, North Carolina State College, Raleigh, North Carolina.

The joint meetings of the Western Section of the American Society of Animal Production and the Western Division of the American Dairy Science Association will be held on the campus of the University of Arizona, Tucson, on July 13 to 15, 1959. The officers of the Western Section for the year 1958–59 are as follows: President, M. E. Ensminger, State College of Washington, Pullman; Vice-President, G. P. Lofgreen, University of California, Davis; Secretary-Treasurer, O. F. Pahnish, University of Arizona, Tucson.

The North Atlantic Section of the American Society of Animal Production will meet at the University of Connecticut, Storrs, Connecticut, August 25 and 26, 1959. Titles for papers to be presented relative to teaching, extension, or research should be sent immediately to Professor G. W. Vander Noot, Department of Animal Husbandry, Rutgers University, New Brunswick, New Jersey.

E. E. Bartley, Kansas State College, George K. Davis, University of Florida and W. P. Lehrer, Jr., University of Idaho, have been appointed to the Editorial Board of the Journal.

Robert L. Blackwell has been appointed Director of the U. S. Sheep Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho, effective January 5, 1959. Dr. Blackwell has been Associate Professor of Animal Husbandry at New Mexico A and M. He received his B.S. degree from New Mexico A and M, his M.S. from Oregon State College, and his Ph.D. from Cornell University. His research has been in animal breeding with sheep and beef cattle. Dr. Blackwell will replace Julius E. Norby who retired recently.

Neil W. Hilston was named Dean of the College of Agriculture and Director of the Agricultural Experiment Station at the University of Wyoming on October 9, 1958. Dr. Hilston joined the staff of the Animal Production Department in 1945 and became head of the Department in 1949. His present appointment fills the vacancy created last June when Dr. Hilton M. Briggs resigned to become President of South Dakota State College.

Mr. E. G. Godbey, Animal Husbandman at Clemson College retired December 31, 1958.

Edgar P. Young, who received his Ph.D. degree from Ohio State University in 1958, has been appointed Assistant Professor of Animal Husbandry at the University of Maryland. He has charge of the swine teaching and research program. He succeeds Dr. Francis C. Wingert who resigned September 1 to accept a position as head of swine research at Cargill-Nutrena research farm, Elk River, Minnesota.

E. S. E. Hafez, Visiting Professor (Rockefeller Foundation appointment) at Washington State College has joined the Animal Science staff as Associate Professor. He received his Ph.D. at the University of Cambridge, England, and did his post-doctorate work at Worcester Foundation of Experimental Biology, Shrewsbury, Massachusetts, University of Cairo, Egypt, and "Lazzaro Spallanzani" Institute for Experimental Spermatology, Milano, Italy. He will be engaged in teaching and research in reproduction and physiology.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. W. D. Gallup, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma, or to the publishers: Boyd Printing Company, Inc., 49 Sheridan Ave., Albany 10, N. Y.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. H. H. Stonaker, Department of Animal Husbandry, Colorado State University, Fort Collins, Colo., or to the publishers, Boyd Printing Company, Inc., 49 Sheridan Ave., Albany 10, N. Y.